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# Diglyceride-rich oils from glycerolysis of edible vegetable oils

C.A. Ferretti, M.L. Spotti, J.I. Di Cosimo*

*Catalysis Science and Engineering Research Group (GICIC), INCAPE, UNL-CONICET, CCT CONICET Santa Fe, Colectora Ruta Nac. 168, km 0, Paraje "El Pozo", 3000 Santa Fe, Argentina*

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ABSTRACT

The human metabolism of 1,3-diglyceride is believed to occur through a pathway that avoids re-synthesis of triglycerides and the consequent fat deposit in the body tissues. The synthesis of healthy vegetable oils with reduced triglyceride content was investigated using food production-compatible MgO as basic catalyst. The partial conversion of the triglycerides present in commercial edible oils into diglycerides was carried out by glycerolysis. After optimizing the reaction conditions such as temperature, glycerol/triglyceride ratio, and inert gas flow rate, triglyceride conversions of up to 70% were reached after 8 h as well as a product containing 54% of diglycerides, 67% of which is the healthier 1,3-isomer.

## 1. Introduction

Nowadays, there is a worldwide interest in designing healthy foods. Foods with low cholesterol, sodium, trans or saturated fats are now readily available in the market.

Vegetable oils contain triacylglycerols (triglycerides, TG) of fatty acids such as oleic, linoleic, linolenic, palmitic and stearic. In particular, oleic, linoleic and linolenic fatty acids are the healthiest since they contain 1, 2 and 3 unsaturations (C=C bonds) in the molecule, respectively. However, a diet rich in lipids that contain TG results in body fat accumulation and obesity.

Diacylglycerols (diglycerides, DG) are minor natural components of various edible oils. Also, they are widely used in foods as emulsifiers since they combine in the molecule a hydrophilic head and a hydrophobic tail that help hydrophilic and lipophilic substances mix together. Examples of DG use in foods are the oil-in-water type emulsions such as mayonnaise and salad dressings and the water-in oil type emulsions represented by margarine, spreads, butter cream fillings, and icings used in baking and the confectionery industry [1]. Other food applications comprise cocoa butter substitutes [2] and milk fat analogues [3,4]. In addition, DG are used as building blocks in the synthesis of structured lipids or in pharmaceutical and cosmetic formulations [5].

DG are esters of glycerol in which two of the hydroxyl groups are esterified with long-chain fatty acids. Esterification can take place at any of the three positions of the glycerol backbone giving rise to different stereochemical forms, *sn*-1,2(2,3)-DG and *sn*-1,3-DG.

Recently, several studies have shown that compared with TG, the regular intake of oil diglycerides (DG), in particular the 1,3-DG isomer, has a positive effect in preventing bodyweight gain and obesity-related diseases such as diabetes, hypertension, stroke, gallbladder disease, some types of cancer and cardiovascular disorders [6,7].

The beneficial effects of the 1,3-DG consumption are believed to be related to its distinct metabolic pathway in the small intestine compared to TG or 1,2-DG rather than to a reduced energy content [7–10]. Whereas TG and the asymmetric 1,2-DG isomer are hydrolyzed to 2-monoglycerides (2-MG) and fatty acids which are immediately resynthesized to TG, the metabolism of the 1,3-DG isomer forms 1(or 3)-MG and fatty acids thereby avoiding the 2-MG pathway toward TG and the consequent fat deposit in the body tissues.

Nowadays, the worldwide biodiesel production totals about 21 million tons per year. Major feedstocks are canola, sunflower and soybean oils. During biodiesel synthesis by oil transesterification, glycerol (Gly) is obtained as the main co-product representing ≈10% of the biodiesel production. Therefore, this industry generates a Gly surplus that is becoming a matter of environmental and economic concern. Thus, new applications intended to convert glycerol into novel products are necessary not only to improve the overall economy of the biodiesel production but also for ecological reasons. Glycerolysis of oils, fats or fatty acid methyl esters are attractive options to transform this biomass-derived compound into functionalized foods or fine chemicals.

1,3-DG can be synthesized by different routes such as glycerolysis of TG, esterification of fatty acids or fatty acid anhydrides with glycerol, hydrolysis of TG followed by esterification or by a combination of methods [1,11,12]. Glycerolysis of TG is preferred from a practical implementation point of view since this is a simple process where oil

* Corresponding author.
*E-mail address:* dicosimo@fiq.unl.edu.ar (J.I. Di Cosimo).

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can be used directly without the splitting to liberate the free fatty acids (FFA). However, this method gives products with a distribution of fatty acids. Thus, to obtain glycerides containing a particular fatty acid profile, selective esterification of fatty acids would be more advisable [5].

Glycerolysis of TG toward DG has been shown to be promoted by enzymes [13,14,11], ionic liquids [15], alkali metal hydroxides, alkoxides and hydrides [16–18]. Enzymatic glycerolysis entails problems derived from the high cost of the enzymes and from the low reaction temperature that might not produce the 1,3-DG isomer in high yields. Homogeneous catalysis and the used of organic solvents, on the other hand, pose catalyst neutralization and separation issues that might not be compatible with food production. All the processes of the patent literature require numerous synthesis and purification steps and, in some cases, the use of the expensive molecular distillation. In addition, large scale preparation of 1,3-DG by conventional chemical methods or by the enzymatic approach imply problems associated to the stability of the unsaturated fatty acid chains and isomerization by acyl migration during synthesis or purification procedures [4,19].

Heterogeneous catalysis using solid catalysts presents the advantage that the catalyst is not dissolved in the reaction medium thereby simplifying the catalyst separation and recycling and shortening the product purification steps. Solid catalysts have been widely applied to glycerolysis reactions [20,21] but these previous works focused on monoglyceride production. In 2014 Zhong et al. [22] reported the use of a KOH-promoted MgO to produce DG by glycerolysis of TG in acetone; a total DG content of 42 wt.% was claimed without providing the concentration of the 1,3-DG isomer in the product.

Here we report our investigations on the glycerolysis of vegetable oils under heterogeneously catalyzed conditions using MgO as a solid catalyst. The process comprises the transesterification of the oil triglycerides with glycerol; the expected overall reaction proceeds according to Eq. (1).

$$2\ \text{TG} + \text{Gly} \leftrightarrow 3\ \text{DG} \quad (1)$$

However, many additional reactions might also occur with formation of other products such as MG and FFA, as well as product interconversion reactions. Thus, we investigated the complex reaction pathways that participate in the synthesis, and the effect of the experimental variables such as temperature and Gly/TG ratio on the shift of the reaction pathways and therefore, on the final glycerolysis product composition. Furthermore, we studied the conditions (temperature and presence of free fatty acids) that affect not only the catalyst and glycerolysis product stability but also the diglyceride isomerization reaction. In particular, we focused on the reaction conditions that favor the synthesis of the 1,3-DG isomer so that the product is a diglyceride-rich healthy edible oil.

## 2. Experimental

### 2.1. Catalyst preparation and characterization

Magnesium oxide was prepared from low-surface area commercial MgO (Carlo Erba 99%; 27 $m^2/g$) by hydration with distilled water. Details are given elsewhere [23]. Excess of water was removed by drying at 353 K overnight. The resulting $Mg(OH)_2$ was decomposed in a $N_2$ flow to obtain high-surface area MgO which was then stabilized for 18 h in $N_2$ at 773 K. Sample was finally ground and sieved, and the particles with average particle size of 177–250 μm were used for the catalytic experiments.

BET surface area was measured by $N_2$ physisorption at 77 K in a NOVA-1000 Quantachrom sorptometer. By X-ray diffraction (XRD), the structural properties of the MgO catalyst was investigated; a Shimadzu XD-D1 diffractometer equipped with Cu-Kα radiation source (λ = 0.1542 nm) was used.

Catalyst basic properties were measured by combining temperature-programmed desorption (TPD) and infrared spectroscopy (FTIR) of $CO_2$. For the TPD experiments, samples were exposed to a flowing mixture of 3% of $CO_2$ in $N_2$ until surface saturation. After removing weakly adsorbed $CO_2$ with a $N_2$ flow, the temperature was increased to 773 K at a ramp rate of 10 K/min. Desorbed $CO_2$ was converted in $CH_4$ on a methanation catalyst (Ni/Kieselghur), and then analyzed using a flame ionization detector (FID). The total base site number ($N_b$, μmol/g) was measured by integration of TPD curve of the evolved $CO_2$. The chemical nature of adsorbed surface $CO_2$ species was determined in previous works by FTIR after $CO_2$ adsorption at room temperature and sequential evacuation at increasing temperatures [24,25].

### 2.2. Catalytic tests

The glycerolysis of vegetable oils with glycerol (Aldrich, 99.0%,) was carried out at 483–513 K in a seven-necked cylindrical glass reactor equipped with a condenser and mechanical stirring. Commercial soybean and sunflower cooking oils (Molinos Río de la Plata S.A.) were used as a source of triglycerides (TG). The 4-phase reactor was operated in a batch regime for the liquid and solid phases at atmospheric pressure. A continuous flow of $N_2$ (70 mL/min) was used to maintain an inert atmosphere inside the reactor.

The catalyst was pretreated ex-situ in $N_2$ at 773 K overnight and then kept at 373 K in a $N_2$ flow until utilization. Oil and glycerol (Gly) were loaded in the reactor and heated at reaction temperature under stirring (700 rpm) using mixtures with Gly/TG molar ratios in the range of 0.5-1.6. The catalyst was then added to start the reaction. Eleven samples of the reaction mixture were extracted during the 8 h catalytic run.

Oil conversion ($X_{TG}$), was calculated by Eq. (2), where $n_{TG}^0$ and $n_{TG}$ are the moles of oil at $t = 0$ and $t = t$, respectively:

$$X_{TG}\ (\%) = \frac{n_{TG}^0 - n_{TG}}{n_{TG}^0} \times 100 \quad (2)$$

### 2.3. Assay of glycerides by HPLC

The HPLC analysis of glycerides was conducted in a Shimadzu LC-10A liquid chromatograph (Shimadzu Corp.) equipped with a quaternary pump system, an on-line degasser, a 20 μL sample loop, column oven, and a spectrophotometer with ultraviolet detector (UV). A 150 × 3.9 mm ID reversed-phase analytical column C18 (μBondapack C18, Waters) was used. The composition of the mobile phase was: mobile phase A, acetonitrile:methanol (4:1 V/V); mobile phase B, *n*-hexane:2-propanol (8:5 V/V). Solvent flow-rate and oven temperature were selected as 0.7 mL/min and 323 K, respectively. Sample solutions for the HPLC assay were prepared in mobile phase A, with a concentration of about 1 mg/mL. Samples were sonicated for 5 min, homogenized and filtered by a 0.22 μm nylon filter. Using this procedure, MG are quantified as a single peak while DG appear as three groups of chromatographic peaks corresponding to palmitic, oleic and linoleic acid; each group contains the 1,2- and 1,3-DG isomers. TG appear in a group of five peaks. For the quantification of glycerides, the corresponding peaks were integrated separately, and the concentrations were calculated using previous calibration. The total glyceride concentration was then calculated by adding up the concentration of individual glycerides. The 1,2- and 1,3-DG compositions determined as described above were confirmed using AOCS official method Cd 11d-96 [26]; similar results were obtained with both procedures.

### 2.4. Determination of free fatty acids (FFA)

Free fatty acids were quantified in the fresh oil and in the oily phase at the end of the 8 h catalytic run using the Lowry and Tinsley method [27]. A homogeneous sample (10 mg) was dissolved in 5 mL of

cyclohexane. Then 1 mL of cupric acetate reagent (pH=6.2) was added and the biphasic system was shaken for 2 min. After centrifugation for 5 min at 2500 RPM, the upper layer was read at 700 nm in a UV–vis Lambda 20 spectrophotometer (Perkin-Elmer). The sample concentration was calculated as wt.% oleic acid based on a standard calibration curve. The results were verified by titration using the AOCS official method Ca 5a-40 [28]. The standard of oleic acid was synthesized by alkaline hydrolysis of glyceryl trioleate (Sigma-Aldrich, 65%) according to the procedure of Wang et al. [29].

### 2.5. Fatty acid composition of glycerides

The fatty acid distribution in the fresh oil and in the glycerides present in the oily phase obtained at the end of the 8-h catalytic test was analyzed. During the analysis, the glyceride-containing samples were converted to fatty acid methyl esters (FAME) by transesterification; about 20 mg of sample were mixed with 5 mL of *n*-hexane, 0.6 mL of dodecane solution (70 mg/mL in *n*-hexane, standard solution) and 0.03 mL of sodium methoxide solution (2 N in methanol). After sample sonication, 0.3 mL of acetic acid solution (2 N in water) and 1 mL of water were added and shaken. After centrifugation at 2500 RPM for 5 min, 1 µL of the organic phase was analyzed with a SRI 8610C gas chromatograph (SRI Instrument) equipped with an on-column injector, a flame ionization detector (FID) and a Supelcowax 10 capillary column. Previous calibration was carried out using standard solutions of the FAME corresponding to the main fatty acids present in the oils.

The fatty acids were coded as (m:n) where m is the carbon chain length and n is the number of unsaturations. They were identified as palmitic (16:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) acids.

## 3. Results and discussion

### 3.1. Catalyst characterization

The MgO catalyst calcined at 773 K was analyzed by XRD showing the characteristic periclase structure (ASTM 4-0829). The BET surface area of the solid was 193 $m^2/g$ with a pore volume of 0.36 mL/g and an average pore diameter of 64 Å.

The solid basic properties were measured by TPD of $CO_2$. The $CO_2$ desorption rate as a function of the desorption temperature is shown in Fig. 1. A $N_b$ value of 656 µmol/g was calculated by integration of the TPD curve. This value reasonably agrees with those of other MgO samples we used in the past [30] to promote different base-catalyzed reactions, as well as with those of MgO catalysts reported in the literature and prepared by different techniques [31,32]. The broad temperature range for $CO_2$ desorption indicates that MgO contains basic sites that bind $CO_2$ with different strength. In previous works we discussed that basic sites of MgO are oxygen-containing surface species and we identified the chemical nature and strength of the different sites: weak (OH groups), medium (oxygen in Mg-O pairs) and strong (coordinatively unsaturated oxygen anions, $O_{cus}$) base sites [33]. By deconvolution of the TPD curve of Fig. 1, the number of the three sites was calculated. Results show a high contribution of medium (239 µmol/g, blue curve) and strong (315 µmol/g, magenta curve) sites, whereas OH groups represent just 15% of the total basicity (102 µmol/g, red curve).

### 3.2. General features of the vegetable oil glycerolysis by heterogeneous catalysis

The solid MgO catalyst was tested in the diglyceride synthesis reaction. The complex reaction system for the glycerolysis reaction consists of four phases: the catalyst solid phase, the $N_2$ phase and two liquid phases, the bottom liquid layer formed by Gly and the "oily phase" containing the oil and the glyceride products located on top of the latter. Gly is slightly soluble in TG but TG is not soluble in Gly.

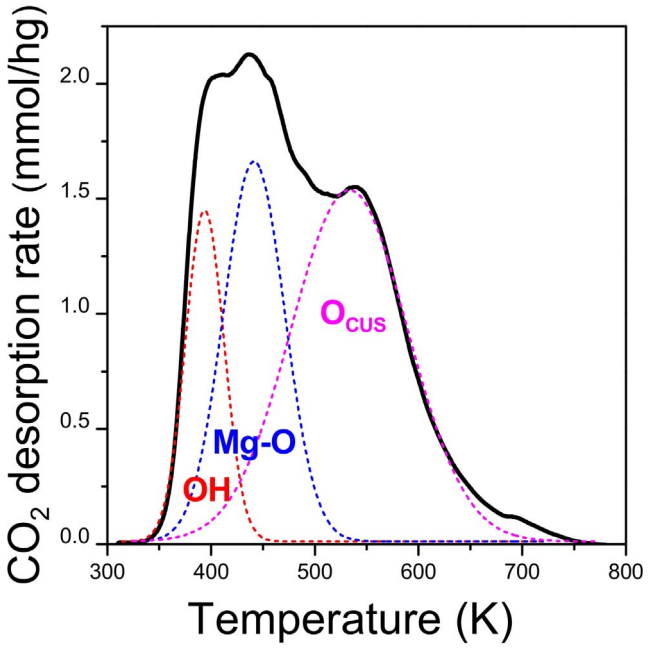


**Fig. 1.** TPD of $CO_2$ on MgO showing the contribution of weak, medium and strong base sites.

Thus, the reaction occurs in the oily phase where both reactants may coexist and where Gly is highly dispersed as small droplets. Only 4 wt.% of Gly is soluble in TG at room temperature and therefore, the glycerolysis reaction must be conducted at higher temperatures to increase the Gly solubility [19]. For the reaction to occur, hydrophilic Gly must be transferred to the hydrophobic oily phase where is consumed. As the reaction proceeds and glyceride products with amphiphilic properties are formed, the Gly solubility in the oily phase increases.

Two MgO-promoted preliminary blank tests were carried at 493 K by loading the reactor in the first experiment with pure glycerol and with pure oil in the second. No conversion was detected in any of the experiments. Another blank test was carried out at 493 K by loading the reactor with the glycerol-oil mixture but without a solid catalyst. No thermal conversion was observed.

Analysis of the sunflower and soybean oil glycerides before reaction, Table 1, entries 1 and 5, respectively, showed that both MG and DG are present in the oils in low contents. A typical catalytic test for the glycerolysis of sunflower oil on MgO is shown in Fig. 2A. A TG conversion of 62% was reached at the end of the 8 h run and MG (15.3 wt.%) and DG (49.3 wt.%) were the only products detected at 493 K. The analysis of the fatty acid distribution in the glycerides after the catalytic experiment of Fig. 2 indicates no significant changes after glycerolysis at 493 K (Table 1, entry 3) compared with the fresh oil (Table 1, entry 1). Similar conclusions can be drawn after the glycerolysis of soybean oil (entries 5 and 6).

Prior to study further the glycerolysis reaction, the presence of external and internal heat and mass transfer limitations was investigated. In order to do so, from the TG curve of Fig. 2A the initial TG conversion rate ($r^0_{TG}$) was calculated from Eq. (3):

$$r^0_{TG} = \left(\frac{n^0_{TG}}{W_{cat}}\right) \times \left(\frac{dX_{TG}}{dt}\right)_{t=0} \quad (3)$$

where $n^0_{TG}$ stands for the moles of oil initially loaded in the reactor and Wcat is the catalyst load. A $r^0_{TG}$ value of 19.8 mol/h kg was calculated. Also, the calculated standard reaction enthalpy ($\Delta H^0_R$) of the glycerolysis reaction of Eq. (1) was ≈0 kcal/mol, indicating that the reaction is thermoneutral, as usually expected for transesterification reactions. Thus, heat transfer limitations can be ruled out [34].

**Table 1**
Composition of fresh oils and glycerolysis products.

| Entry | Oil | Gly/TG | Composition (wt.%) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Fatty acid distribution | | | | Glyceride content | | | | | FFA |
| | | | 16:0 | 18:1 | 18:2 | 18:3 | MG | DG | TG | 1,2-DG | 1,3-DG | |
| 1 | sunflower | – | 7.1 | 29.9 | 63.0 | – | 1.1 | 1.6 | 97.3 | 0.4 | 1.2 | 0.04 |
| 2 | sunflower[a] | 0.5 | 6.6 | 30.5 | 62.9 | – | 13.6 | 42.4 | 44.0 | 12.0 | 30.4 | 2.9 |
| 3 | sunflower[b] | 0.6 | 7.7 | 36.6 | 55.7 | – | 15.3 | 49.3 | 35.4 | 16.0 | 33.4 | 2.6 |
| 4 | sunflower[c] | 1.6 | 7.0 | 33.1 | 59.9 | – | 38.4 | 42.1 | 19.5 | 14.6 | 27.5 | 0.9 |
| 5 | soybean | – | 11.1 | 25.2 | 54.8 | 8.9 | 0.6 | 3.4 | 96.0 | 1.0 | 2.4 | 0.02 |
| 6 | soybean[d] | 0.6 | 16.6 | 19.3 | 59.2 | 4.9 | 18.4 | 46.6 | 35.0 | 15.0 | 31.6 | 3.2 |

[a] After experiment of Fig. 4.
[b] After experiment of Fig. 2.
[c] After experiment of Fig. 5.
[d] After glycerolysis at experimental conditions idem entry 3.

Then, the extent of the external mass transfer resistance was evaluated by calculating the Mears number ($\omega$), Eq. (4) [35]:

$$\omega = \frac{r^0_{TG}\rho_p R_p}{k_c C^0_{TG}} < \frac{0.15}{n} \quad (4)$$

where $\rho_p$ is the catalyst particle density (1550 kg/m$^3$), $R_p$ is the average particle radius ($1.25 \times 10^{-4}$ m), $k_c$ is the mass transfer coefficient (0.63 m/h) calculated with $k_c = \frac{ShD_m}{2R_p}$, where $D_m$ ($8.0 \times 10^{-6}$ m$^2$/h) is the molecular diffusion coefficient calculated from the Wilke-Chang correlation [36], $Sh$ is the Sherwood number calculated with the Frösslings equation ($Sh = 2 + 0.55Re^{0.5}Sc^{0.33}$), $Re$ is the Reynolds number according to the Kolmogoroffs theory ($Re = \frac{\rho_L d_p^{4/3}\varepsilon^{1/3}}{\mu_L}$), where ρL is the liquid density in kg/m$^3$; $d_p$ is particle size in m, ε is the average dissipation energy of the stirrer in m$^2$/s$^3$ and μL is the liquid viscosity in kg/m s, and $Sc$ is the Schmidt number ($\frac{\mu_L}{\rho_L D_m}$) [37–42], $C^0_{TG}$ is the initial TG concentration (1040 mol/m$^3$) and $n$ is the reaction order taken as 1. A $\omega$ value of $6 \times 10^{-3}$ was calculated thereby confirming the absence of interparticle diffusion limitations.

To verify the absence of intraparticle mass transfer limitations, the Weisz-Prater criterion [43] for a first order reaction and spherical catalyst particles was used, Eq. (5):

$$\Phi = \frac{r^0_{TG}\rho_p R_p^2}{D_{eff} C^0_{TG}} < 1 \quad (5)$$

where $\Phi$ is the dimensionless Weisz-Prater modulus, $D_{eff}$ is the effective diffusion coefficient ($4.6 \times 10^{-6}$ m$^2$/h) calculated with $D_m$ and using a particle porosity of 0.57. A value of 0.1 was calculated for $\Phi$, which corresponds to a Thiele modulus of 0.1 and an effectiveness factor of 0.99. This result confirms the absence of pore diffusion limitations.

Then, no internal or external mass transfer diffusional limitations seem to be present under typical reaction conditions and the reaction takes place under kinetic control.

### 3.3. Reaction pathways for the synthesis of diglycerides on MgO

It has been discussed in the literature that glyceride formation by glycerolysis of TG occurs stepwise [20,44]; Eq. (6) has been postulated as the first reaction step where DG and MG form in a 1:1 molar ratio. In agreement with that, as can be observed in Fig. 2A, the initial slopes of the MG and DG curves are similar and different from zero indicating that both products form simultaneously with comparable rates directly from TG. In fact, the values of the initial MG and DG formation rates calculated assuming that only Eq. (6) takes place at initial conditions were 9.38 mol/h kg for MG and 9.65 mol/h kg for DG.

$$TG + Gly \leftrightarrow DG + MG \quad (6)$$

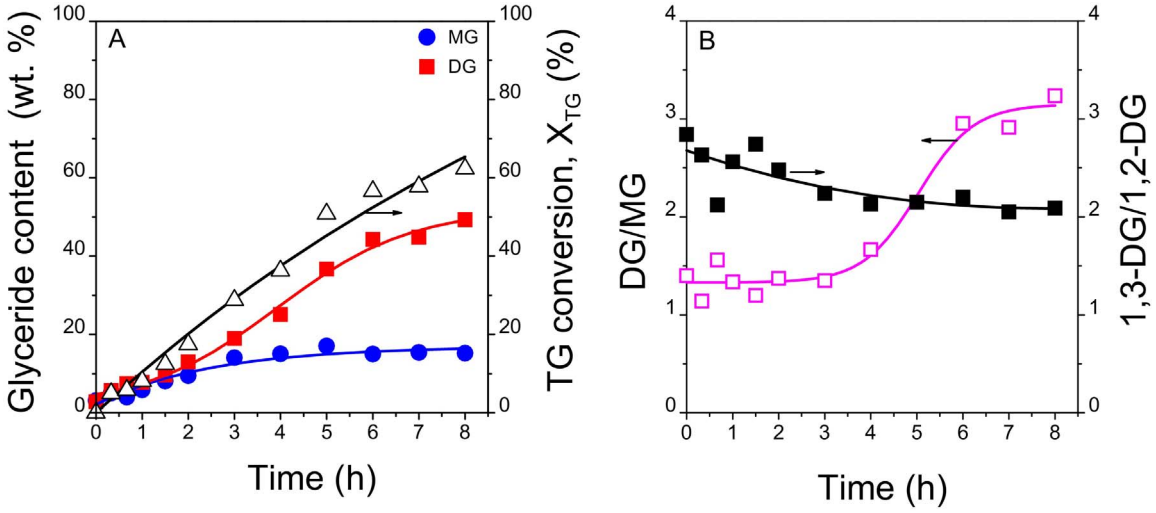


**Fig. 2.** Time evolution of the glycerolysis reaction on MgO. (A) Glyceride content and TG conversion; (B) DG/MG and 1,3-DG/1,2-DG ratios [sunflower oil, T = 493 K, Gly/TG = 0.6 (molar ratio), $W_{cat}/n^0_{TG}$ = 9 g/mol, 70 mL $N_2$/min].

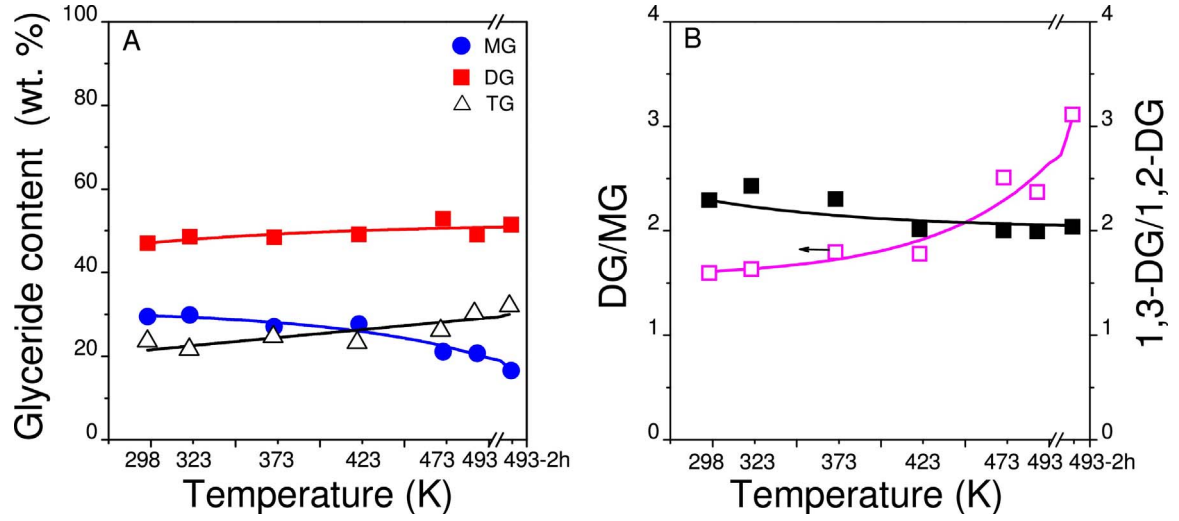


**Fig. 3.** Effect of the addition of fatty acids on a glyceride mixture stirred at increasing temperatures (A) Glyceride content; (B) DG/MG and 1,3-DG/1,2-DG ratios [sunflower oil, see section 3.3].

Different consecutive reaction steps might take place depending on the Gly availability in the reaction zone. In Fig. 2A, the change of the slope of the MG and DG curves after 2 h of reaction suggests glyceride interconversion according to Eq. (7):

$$TG + MG \leftrightarrow 2\ DG \quad (7)$$

The flattening of the MG curve at high reaction times indicates that the MG formation rate by Eq. (6) equals MG consumption by Eq. (7). On the other hand, the higher slope of the DG curve at high reaction times clearly suggests that DG is now formed with a different stoichiometry resulting from Eq. (7). The latter is envisaged in Fig. 2B where the DG/MG ratio is rather constant in the first hours of the reaction in agreement with the prevalence of Eq. (6) and then increases with reaction time, reaching a value of 3.2 at the end of the catalytic run which corresponds to a 49.4 wt.% DG (Fig. 2A).

The overall reaction leading to DG, Eq. (1), results then from the combination of steps (6) and (7). Later on, the minor contribution of esterification reactions to the total DG concentration will be discussed in detail. An excess of Gly over the stoichiometric amount of Eq. (1) would shift the reaction equilibrium to the right with the concomitant enhancement of the DG yield. However, under those conditions Eq. (8) also occurs.

$$DG + Gly \leftrightarrow 2\ MG \quad (8)$$

Thus, to obtain high DG yields, the initial Gly/TG ratio must be selected from a careful balance between Gly availability in the reaction zone and the occurrence of the competitive formation of MG (Eqs. (6) and (8)). Contrarily, when MG are the desired products, an increase of the Gly/TG ratio always works favorably [20].

Two MG isomers are expected to form, one with the acyl chain at terminal positions of the glycerol backbone (1 (or 3)-MG) and the other in the middle (2-MG). Similarly, 1,2 (or 2,3)-DG and 1,3-DG are obtained during the glycerolysis reaction.

Under the reaction conditions of Fig. 2, DG are the main products with the 1,3-DG isomer being the main component (33.4 wt.% at the end of the run). Therefore, the valuable 1,3-DG having the acyl chains at terminal positions can be obtained in high yields by glycerolysis of sunflower oil on MgO at 493 K. Recently, we obtained similar results during the synthesis of MG by glycerolysis of methyl oleate with glycerol on MgO. We found that the isomers having terminal acyl chains (1 (or 3)-MG), that result from dissociation of terminal OH groups in the glycerol molecule, form in much higher selectivities, despite the fact that dissociation of the secondary OH group of Gly (and formation of a 2-glyceroxide species) is energetically favored. This controversy was explained by using theoretical calculations that demonstrated that indeed a 2-glyceroxide species reacts with the methyl oleate molecule forming both, 1 (or 3)-MG and 2-MG isomers. Two transition states and a tetrahedral intermediate (TI) are involved in the formation of both MG isomers. However, the pathway toward 2-MG is limited by sterical effects associated to the TI whereas the TI leading to 1 (or 3)-MG is relatively easy to form [25,45], thereby explaining its higher selectivities.

Although 1-MG and 1,3-DG are the main components of the MG and DG fractions, respectively, isomer interconversion might take place by acyl migration. In particular, for diglycerides, the reaction occurs according to Eq. (9):

$$1,2\text{-DG} \leftrightarrow 1,3\text{-DG} \quad (9)$$

The rate of intramolecular rearrangement by acyl migration depends on temperature, solvent, length and nature of the fatty acid chains as well as on the catalyst acid-base properties [46,47]. In the experiment of Fig. 2, both isomers were present from the beginning of the reaction; Fig. 2B shows that the 1,3-DG/1,2-DG isomer ratio was always higher than 2 and slightly decreased as the reaction proceeded. Thus, in spite of the fact that Eq. (9) is reversible, the equilibrium is shifted to the right and the most stable isomer 1,3-DG prevails at 493 K. After 4 h of reaction the isomer ratio remained constant at $\approx 2.1$, which is the value expected at equilibrium conditions [47–49].

In the presence of traces of water, TG, DG and MG may undergo hydrolysis with formation of free fatty acids (FFA). These reactions are detrimental to the stability of the glycerolysis products and affect the total DG content and the 1,3-DG/1,2-DG ratio during storage [50]. In fact, the results of Table 1 show an increase of the FFA content at the end of the catalytic runs (entries 2–4 and 6) compared with the fresh oils (entries 1 and 5). However, the values are similar to those reported by Corma et al. for the synthesis of MG at 513 K [20].

To illustrate the effect of the presence of FFA on the product distribution, an additional experiment was carried out. The liquid phases of several catalytic experiments were mixed together at room temperature; the mixture contained 29 wt.% MG, 47 wt.% DG, 24 wt.% TG and 0.3 wt.% FFA. Then, 1 wt.% of oleic acid was added and the mixture was heated from 298 to 323, 373, 423, 473 and 493 K under stirring and kept 1 h at each temperature. After reaching 493 K another 1 wt.% of oleic acid was added and the mixture was stirred for 2 more hours; the results are presented in Fig. 3. Previously, another experiment at increasing temperatures was performed with the same glyceride mixture but without the addition of oleic acid; changes in the glyceride composition were negligible indicating that the glycerolysis

products are stable when heated at increasing temperatures. Thus, the results of Fig. 3 must be ascribed to the addition of oleic acid; MG decreased with temperature at the expense of TG, whereas DG slightly increased (Fig. 3A). Thus, the DG/MG ratio gradually increased (Fig. 3B). An explanation for these results is the conversion of MG by esterification through Eq. (10):

$$MG + 2\ FFA \leftrightarrow TG + 2H_2O \quad (10)$$

which could take place stepwise by Eqs. (11) and (12):

$$MG + FFA \leftrightarrow DG + H_2O \quad (11)$$

$$DG + FFA \leftrightarrow TG + H_2O \quad (12)$$

On the other hand, the 1,3-DG/1,2-DG isomer ratio (Fig. 3B) slightly decreased to reach a constant value at temperatures higher than 423 K. Thus, it seems that fatty acids do not promote the isomerization of DG at high temperatures.

### 3.4. Effect of the Gly/TG ratio

According to the stoichiometry of Eq. (1), formation of DG entails the use of a low reactant molar ratio (Gly/TG = 0.5). However, taking into account that the first reaction step of the overall process, Eq. (6), requires a Gly/TG = 1 and that an excess of glycerol would shift the reaction toward formation of MG, Eq. (8), the optimal Gly/TG ratio should be chosen from a narrow range of values. As explained above, the optimal ratio should provide an acceptable concentration of glycerol in the oily phase for the reaction to occur at high rates, but preventing MG from being the main products.

Several catalytic experiments with sunflower oil and Gly/TG ratios of 0.5, 0.6, 0.8, 1.0, 1.3 and 1.6 were carried out to elucidate the influence of the Gly/TG ratio on the quality of the glycerolysis product. Figs. 4 and 5 show the time evolution for selected experiments with Gly/TG ratios of 0.5 and 1.6, respectively. In the experiment at stoichiometric conditions, Fig. 4A, formation of DG predominates from the beginning of the reaction but the TG conversion rate is low, as indicated by the $X_{TG}$ curve. On the other hand, under excess glycerol, Fig. 5A, the $X_{TG}$ is enhanced but Eq. (6) prevails during the entire run resulting in similar DG and MG concentrations.

The sigmoidal shape of the time evolution of the DG/MG ratio is consistent with the discussion above since for the experiment at stoichiometric conditions (Gly/TG = 0.5, Fig. 4B) the constant initial DG/MG ratio upholds for a shorter period of time compared with the experiment of Fig. 2B. This indicates that Eq. (6) predominates only in the first two hours of the catalytic run for Gly/TG = 0.5 and that at longer times the reaction proceeds through Eq. (7). Contrarily, for the experiment at Gly/TG = 1.6 (Fig. 5B) the constant DG/MG ratio throughout the catalytic run confirms that the reaction mainly occurs according to Eq. (6).

The effect of the Gly/TG ratio on the 1,3-DG/1,2-DG isomer ratio is less evident since similar curves and final values were obtained regardless of the reactant ratio (Figs. 2 B, 4 B and 5 B).

Fig. 6 summarizes the results at the end of the 8 h runs for all the Gly/TG ratios used. Clearly, the best 1,3-DG concentration (36.4 wt.%), representing 67% of the total DG content (54.2%), was obtained at Gly/TG = 0.8 (Fig. 6A) but the highest DG/MG ratio (≈3.2, Fig. 6B) was attained at reactant ratios close to the stoichiometric conditions of Eq. (1).

As discussed in Section 3.1, MgO contains traces of adsorbed water and surface OH groups that might be responsible for the FFA formation by TG hydrolysis (reverse reaction in Eq. (10)). Table 1 shows the effect of the Gly/TG ratio on the FFA content at the end of the 8 h runs for the experiments of Figs. 2, 4 and 5. The decrease of the FFA concentration with increasing Gly/TG is probably explained by the fact that the enhanced MG concentration at high reactant ratios reduces the extent of the hydrolysis reaction by shifting the equilibrium to the right in Eq. (10).

### 3.5. Effect of the reaction temperature, FFA formation and flow rate of inert gas

The influence of the reaction temperature on the glycerolysis reaction was investigated in the range of 483–513 K using sunflower oil and a Gly/TG ratio of 0.8. Fig. 7 shows the results for the experiment at 513 K and Table 2 (entries 1–4) summarizes the glycerolysis product composition at the end of the 8 h runs at 483–513 K. The MG content decreased with increasing the temperature but this was not accompanied by a concomitant increase of DG. In fact, the highest total DG and 1,3-DG contents were obtained at 493 K, whereas the 1,3-DG/1,2-DG ratio was almost independent of the temperature.

It is well known that the reaction temperature affects both, the glycerol solubility in the oil and the reaction kinetics that follows an Arrhenius law. At room temperature the solubility of Gly is low (4–5 wt. %) but increases to ≈50% at 523 K [44]. Thus, higher temperatures enhance the effective Gly concentration in the oily phase favoring the glycerolysis kinetics.

The overall effect of temperature was evaluated calculating the initial reaction rates ($r^0_{TG}$) with Eq. (3); results are presented in Table 2. The $r^0_{TG}$ values doubled upon a temperature increase of just 30 K

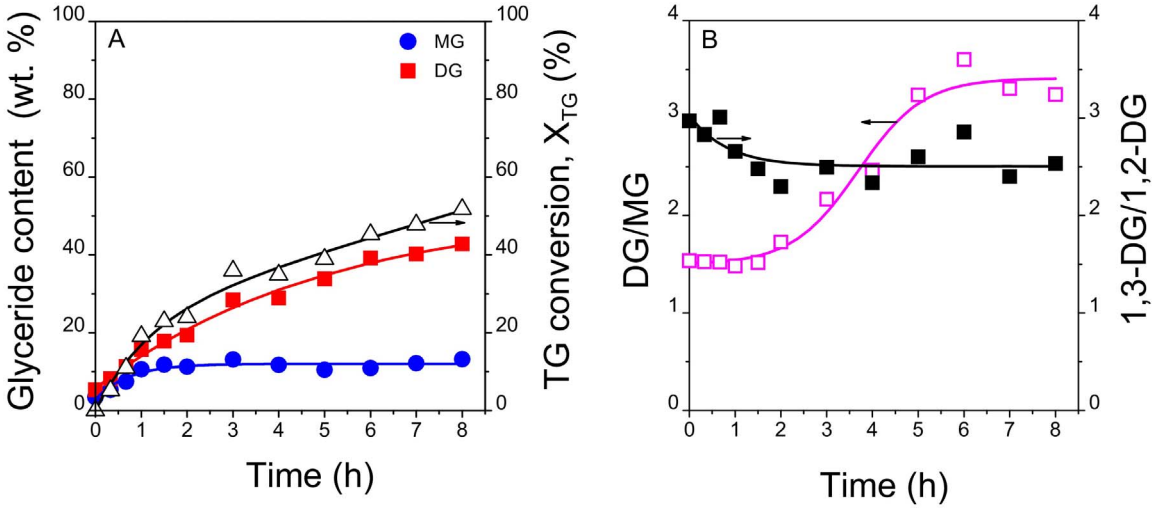


**Fig. 4.** Time evolution of the glycerolysis reaction on MgO. (A) Glyceride content and TG conversion; (B) DG/MG and 1,3-DG/1,2-DG ratios [sunflower oil, T = 493 K, Gly/TG = 0.5 (molar ratio), $W_{cat}/n^0_{TG}$ = 9 g/mol, 70 mL $N_2$/min].

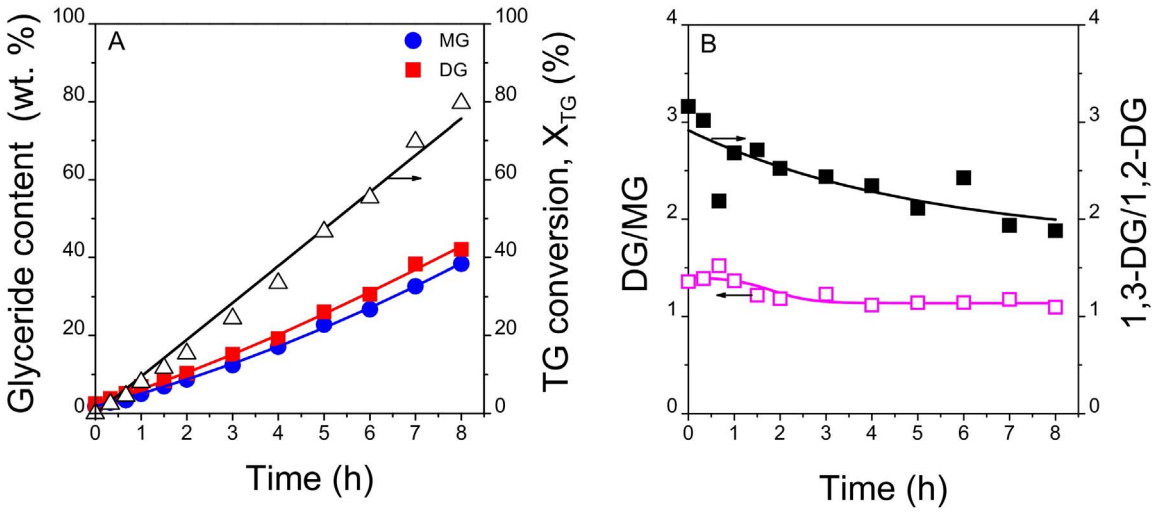


**Fig. 5.** Time evolution of the glycerolysis reaction on MgO. (A) Glyceride content and TG conversion; (B) DG/MG and 1,3-DG/1,2-DG ratios [sunflower oil, T = 493 K, Gly/TG = 1.6 (molar ratio), $W_{cat}/n^0_{TG}$ = 9 g/mol, 70 mL $N_2$/min].

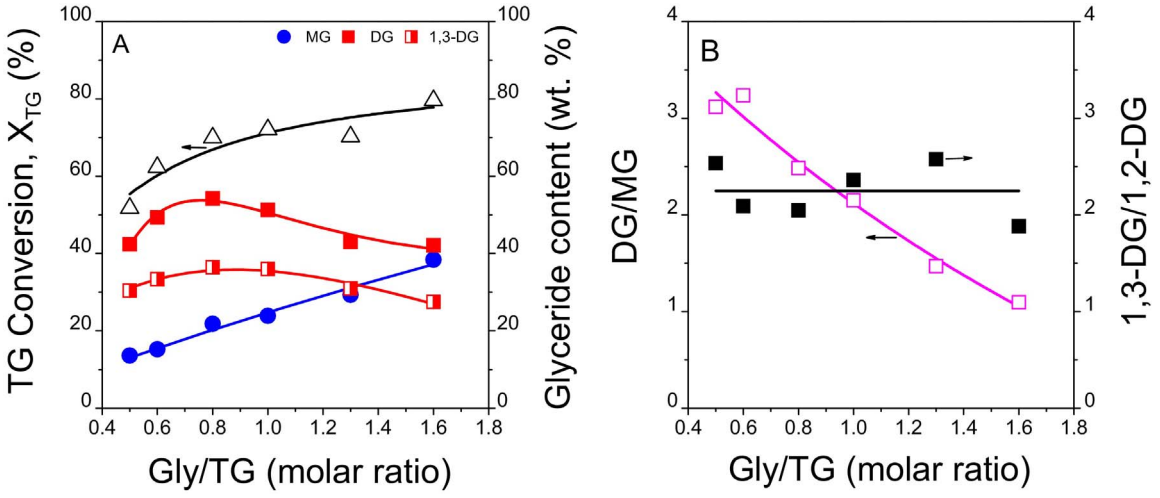


**Fig. 6.** Effect of the Gly/TG ratio on the glycerolysis reaction on MgO. (A) Glyceride content and TG conversion; (B) DG/MG and 1,3-DG/1,2-DG ratios [sunflower oil, T = 493 K, 8 h, $W_{cat}/n^0_{TG}$ = 9 g/mol, 70 mL $N_2$/min].

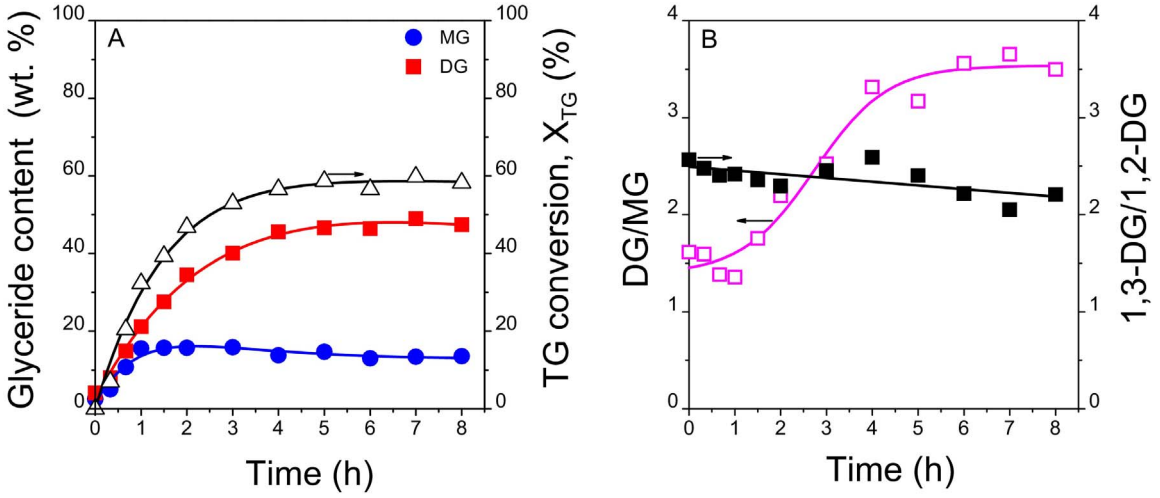


**Fig. 7.** Time evolution of the glycerolysis reaction on MgO. (A) Glyceride content and TG conversion; (B) DG/MG and 1,3-DG/1,2-DG ratios [sunflower oil, T = 513 K, Gly/TG = 0.8 (molar ratio), $W_{cat}/n^0_{TG}$ = 9 g/mol, 70 mL $N_2$/min].

**Table 2**
Effect of the reaction temperature on the glycerolysis reaction.

| Entry | Temperature (K) | $r^0_{TG}$ (mol/h kg) | Composition at 8h | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | $X_{TG}$ (%) | Glyceride content (wt.%) | | | DG/MG | 1,3-DG/1,2-DG | FFA (wt.%) |
| | | | | MG | DG | 1,3-DG | | | |
| 1 | 483 | 21.8 | 65.2 | 22.9 | 45.3 | 30.7 | 2.0 | 2.1 | 1.0 |
| 2 | 493 | 29.3 | 69.9 | 21.8 | 54.2 | 36.4 | 2.5 | 2.0 | 1.6 |
| 3 | 503 | 45.8 | 62.8 | 13.8 | 51.5 | 35.6 | 3.7 | 2.2 | 3.7 |
| 4 | 513 | 50.3 | 58.2 | 13.6 | 47.4 | 32.6 | 3.5 | 2.1 | 3.6 |
| 5 | 503[a] | 45.5 | 19.5 | 3.3 | 27.6 | 19.3 | 8.4 | 2.3 | 8.8 |
| 6 | 503[b] | 45.8 | 23.0 | 10.2 | 18.0 | 10.7 | 1.8 | 2.3 | 0.7 |
| 7 | 513[c] | 51.9 | 58.4 | 14.5 | 48.4 | 33.3 | 3.3 | 2.2 | 6.6 |

[Sunflower oil; Gly/TG = 0.8; $W_{cat}/n^0_{TG}$ = 9 g/mol; 70 mL $N_2$/min].
[a] 1 wt.% of oleic acid added at t = 0.
[b] Experiment of entry 3 at t = 0.67 h.
[c] 35 mL $N_2$/min.

(483–513 K). An activation energy of 14.6 kcal/mol was then calculated using the Arrhenius equation. This value is similar to that calculated by Noureddini et al. [51] (13.1 kcal/mol) for the solvent-free homogeneously catalyzed transesterification of TG with methanol promoted by sodium hydroxide at 303–343 K, but almost triples that reported by Kumoro [52] (5.5 kcal/mol) for the glycerolysis of TG under reaction conditions similar to the latter but using 2-propanol as a solvent. On the other hand, Corma et al. [20] measured 20 kcal/mol for the heterogeneously catalyzed TG glycerolysis toward MG at 473–513 K.

Despite the positive effect on the initial reaction rate, higher temperatures also favored formation of FFA and other undesired products that increase the final product color, Table 2. In addition to being detrimental to the product quality, these compounds affect negatively the solid catalyst stability causing deactivation at high reaction times. The latter can be observed in Table 2 since the $X_{TG}$ values at the end of the 8 h runs decreased above 493 K. Fig. 7 illustrates these facts for the reaction conducted at 513 K showing that the TG conversion curve levels off after 3 h. The FFA formation in the course of the high temperature reaction and the consequent acid poisoning of the catalyst basic sites, together with the onset of Eq. (10), explain the results at high reaction times of Fig. 7 and Table 2.

The glycerolysis product stability after adding FFA without a solid catalyst, was studied in section 3.3 (Fig. 3) but in view of the results at high reaction times of Table 2 (entries 1–4) and Fig. 7, the effect of the presence of FFA was further investigated but now during the catalytic reaction. An additional experiment was carried out at 503 K in which 1 wt.% of oleic acid was added to the reactants (Table 2, entry 5) and the results were compared with the experiment without FFA addition (entry 3). Similar $r^0_{TG}$values were calculated for both runs. This indicates that the effect of the FFA addition on the shift of the reaction pathways or on poisoning the MgO active sites was negligible at the beginning of the reaction. However, in the presence of FFA the TG concentration started increasing after 3 h because of TG formation by Eq. (10); the MG concentration concomitantly decreased. Thus, the addition of FFA stopped the reaction and a lower $X_{TG}$ was measured at the end of the run compared to the experiment without acid addition. Both experiments were further compared at similar $X_{TG}$ values (entries 5 and 6). In the catalytic test with FFA addition, the consumption of MG by Eq. (10) and formation of DG by Eq. (11) led to a much higher DG/MG ratio. On the other hand, the 1,3-DG/1,2-DG ratios were similar for both experiments indicating that free fatty acids do not catalyze the isomerization of diglycerides, thereby confirming the results of Fig. 3B.

Finally, the flow rate of the inert gas ($N_2$) used to maintain an inert atmosphere inside the reactor was found to affect the quality of the glycerolysis products. At 513 K, an experiment using 35 mL/min (Table 2, entry 7) was compared with the experiment of entry 4 (70 mL/min). Although $r^0_{TG}$ and glyceride composition at the end of the run were similar for both experiments, the use of a low flow rate yielded darker products that contained a higher concentration of FFA compared to the standard reaction conditions.

## 4. Conclusions

A diglyceride rich-oil was synthesized by glycerolysis of vegetable oils. Valuable 1,3-diglyceride was obtained as the main diglyceride isomer with concentrations of up to 36 wt.% using a strongly basic MgO catalyst. This catalyst was selected based on our previous experience on the base-catalyzed glycerolysis of fatty acid methyl esters [21,25,33,45]. However the specific catalyst acid-base requirements for the selective synthesis of 1,3-diglyceride is yet to be elucidated.

A narrow range of glycerol/triglyceride ratios can be used for the selective synthesis of diglycerides and to avoid the competitive pathway toward monoglycerides.

Election of the reaction temperature is a trade-off process between the need of high triglyceride conversion rates and formation at higher temperatures of free fatty acids and other undesirable products that are detrimental to the product quality and stability.

Under the present experimental conditions the undesirable isomerization of 1,3-diglyceride toward the 1,2-isomer was shown to be negligible and almost independent of the operational variables.

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