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Low-temperature chemical synthesis of high-purity diacylglycerols (DAG) from monoacylglycerols (MAG)

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Abstract A chemical method was developed for low-temperature synthesis of DAG from MAG followed by an easy purification procedure in order to obtain high-purity DAG. Solvent-assisted and solvent-free reaction conditions were used, combined with different catalysts (sodium methoxide, p-toluenesulfonic acid, methanesulfonic acid, and sulfuric acid). All reactions were performed at 35 and 70 °C. By increasing both acidity and polarity of the catalyst the equilibrium shifts towards the formation of DAG. When using sulfuric acid in solvent-assisted condition at 70 °C, 88% conversion was obtained after 20 min of reaction (77 % w/w DAG in the reaction mixture after evaporation of the solvent). After purifying by means of column chromatography, 96% pure DAG were obtained. The overall yield of DAG was 81%.

Key words high-purity diacylglycerols (DAG), monoacylglycerols (MAG), transesterification, solvent-free, solvent-assisted

Abbreviations and symbols

DAG Diacylglycerol(s)

FFA Free fatty acid(s)

GPC Gel permeation chromatography

MAG Monoacylglycerol(s)

25 MSA Methanesulfonic acid

26 PTSA p-Toluenesulfonic acid

27 SA Solvent-assisted

28 SF Solvent-free

29 SFA Sulfuric acid

30 TAG Triacylglycerol(s)

31 TLC Thin layer chromatography

32 Introduction

33 Monoacylglycerols (MAG) and diacylglycerols (DAG) are widely used as emulsifiers in the food,
34 cosmetic, pharmaceutical and chemical industry [1-3]. Recently DAG caught a lot of attention due to
35 their nutritional properties and dietary effects [1,2,4-6]. Oils with a high DAG content are 'generally
36 recognized as safe (GRAS)' [7] and have been commercialized in the USA [4,6,8] and Japan [4,6,9].
37 Diacylglycerols can be used to improve the quality of meat [10] or suppress crystallization in order to
38 reduce the solid fat content in palm oil [11]. Pure DAG can be used as building blocks for fatty acid
39 derivatives [12] and as starting materials for synthesizing pharmaceutical products [13]. Because
40 traditional chemical methods require high temperatures [1,2,4,6,14] which cause auto-oxidation of
41 unsaturated fatty acids [15], DAG are mainly produced by using enzyme catalysis [1,2,4,9]. Enzymatic
42 production allows more selective production of DAG, resulting in low TAG content [16] while
43 chemical approaches can lead to unwanted TAG (Fig. 1).

44 Some publications address low-temperature, non-enzymatic synthesis of DAG from MAG [17,18],
45 however, the methods, which these publications refer to, are not suitable for large-scale production
46 of DAG. Isai *et al* used 1:640 (w/w) MAG/solvent ratio and 5-10% (w/w) p-toluenesulfonic acid
47 catalyst (based on MAG mass), yielding 12.8×10^{-6} M concentration of DAG [17]. The synthesis by

48 Stamatov *et al* was intended to produce enantiomerically pure products and involved three-step
49 synthesis [18].

50 In the present work we developed a low-temperature highly selective chemical method to produce
51 DAG from MAG followed by an easy purification step, which yielded 96% pure DAG. The method can
52 easily be scaled up to produce large quantities of pure DAG.

53 **Materials and methods**

54 *Materials*

55 Monoacylglycerols were donated by Oleon NV (Oelegem, Belgium), they contained 95.1% MAG, 3.8%
56 DAG, 0.3 % free glycerol and the acid value was 1.6 mg KOH/g. Table 1 shows the fatty acid profile of
57 this product. Sodium methoxide (98% purity) was obtained from Alfa Aesar (Karlsruhe, Germany). p-
58 Toluenesulfonic acid monohydrate ('for analysis' grade) was obtained from Merck Chemicals
59 (Darmstadt, Germany). Methanesulfonic acid (99%), sulphuric acid (96%), acetic acid (100%), ethyl
60 acetate, methanol (99%), tetrahydrofuran (99%), and petroleum ether (40-60 °C) were analytical
61 grade and obtained from Acros Organics (Geel, Belgium). Orcinol (98%) and silica gel for
62 chromatography were obtained from Acros Organics.

63 *Reaction Procedures*

64 Diacylglycerols were formed through intermolecular exchange of acyl moieties in MAG molecules.
65 Transesterification between two MAG results in the formation of one DAG and one glycerol molecule
66 (see Fig. 1a). The reaction was performed both acid- and base-catalyzed, and all reactions were
67 performed at 35 and 70 °C. To shift the equilibrium and increase the DAG yield, petroleum ether was
68 added to the reaction mixture as solvent. Monoacylglycerols were dried under vacuum before use to
69 prevent reaction between water and the sodium methoxide catalyst. All reactions were performed in
70 a three-necked glass flask (250 mL) equipped with a cooler and placed in an oil bath held at constant
71 temperature. The reaction mixture was stirred with a magnetic stirrer. All reactions were performed
72 in duplicate.

73 Sodium methoxide is a good catalyst to perform transesterifications [19]. Thus, as a reference, the
74 reaction was performed by using sodium methoxide at 35 and 70 °C under both solvent-free and
75 solvent-assisted conditions. For solvent-free reactions, 0.033 mol of sodium methoxide per mol MAG
76 was added after heating the MAG. For solvent-assisted reactions, the MAG were mixed with
77 petroleum ether in 1:1 mass ratio. This mixture was heated to the desired reaction temperature
78 before the catalyst was added.

79 For acid-catalyzed solvent-free reactions, 0.088 mol/mol of p-toluenesulfonic acid (dissolved in 1.5
80 mL tetrahydrofuran to increase the solubility and prevent the rate of dissolution to be the limiting
81 factor in reaction kinetics), methanesulfonic acid or sulfuric acid were added after heating the MAG.
82 Samples (5 mL) were collected during the first 60 min of reaction and were immediately treated with
83 2-3 drops of a 7 M potassium carbonate solution to neutralize the catalyst. For solvent-assisted
84 reactions, the same procedure was followed but the MAG were mixed with the solvent in 1:1 mass
85 ratio before heating.

86 *Analysis of the Reaction Mixture*

87 The final reaction mixtures as well as intermediate samples were analyzed by means of gel
88 permeation chromatography (GPC) by using an Agilent 1100 series system (Diegem, Belgium) and
89 isocratic elution with THF on a 300 mm x 7.80 mm, 5 micron, 100 Å Phenogel (Phenomenex, Utrecht,
90 The Netherlands) column connected to a refractive index detector. The common errors for the
91 determination of the amounts of MAG, DAG, and TAG were 1-2% (w/w).

92 *Purification*

93 The reaction mixture was purified by means of column chromatography (500 mm x 50 mm glass
94 column) on silicagel (60-200 µm, 60 Å) by using the following analytical-grade solvents: petroleum
95 ether, ethyl acetate, acetic acid, and methanol [20]. Silica gel was mixed with an appropriate amount
96 of petroleum ether and packed into the column (approx. 350 mm of stationary phase).

97 About 20 g of raw mixture was dissolved in 20 g of petroleum ether and loaded on the column.
 98 Samples were eluted with different solvent mixtures [20] (see Table 2). The amount of solvent was
 99 slightly reduced compared to Zhao et al [20]. Samples (30 mL) were collected and analyzed by TLC.
 100 Each of the collected samples was spotted on a TLC plate for qualitative analysis. On each TLC plate a
 101 sample of crude reaction mixture dissolved in petroleum ether was spotted as a reference. Some
 102 fractions were injected on GPC for identification of the spots. TLC (silica gel 60W plate) was
 103 performed by using 90:10:1 (v/v) petroleum ether/ethyl acetate/acetic acid as the mobile phase.
 104 Visualization was carried out by spraying orcinol solution (0.1% 5-methylresorcinol dissolved in 5%
 105 H₂SO₄) on the TLC-plate and heating it at 110 °C for 10 min [21].
 106 After TLC analysis the samples containing the same components were combined and after
 107 evaporation of the solvent, the pure products were obtained. Pure products were analyzed on GPC
 108 to determine the purity of the obtained products.

109 Results

110 *Transesterification Reactions of MAG*

111 *Base-catalyzed Reactions*

112 Sodium methoxide is a good catalyst for transesterification [19], thus, reference experiments with
 113 sodium methoxide were performed for 60 min at 35 and 70 °C using solvent-free and solvent-
 114 assisted conditions. The grade of conversion of MAG to DAG was calculated according to Eq. 1.

$$115 \text{ Conversion (\%)} = \frac{\text{Actual amount of DAG (\% w/w)}}{\text{Theoretical maximum amount of DAG (\% w/w)}} \times 100\% \quad [\text{Eq. 1}]$$

116 Whereas the theoretical maximum amount of DAG is calculated as follows:

$$117 \text{ Theoretical maximum amount of DAG (\% w/w)} = \frac{(m_{\text{MAG}}/MM_{\text{MAG}}) \times MM_{\text{DAG}}}{m_{\text{reaction mixture}}} \quad [\text{Eq. 2}]$$

118 m_{MAG} = mass of MAG added to the reaction mixture (g)

119 MM_{MAG} = molar mass of MAG (g/mol)

120 MM_{DAG} = molar mass of DAG (g/mol)

121 $m_{\text{reaction mixture}}$ = total mass of the reaction mixture (g)

122 The equilibrium conversion was determined by plotting conversion (Eq. 1) versus time and by
123 nonlinear fitting of the data by using the Prism 6 software from Graphpad Software Inc (La Jolla,
124 California, USA). The equilibrium amounts were the plateau values of the fitted curves. For alkali-
125 catalyzed solvent-free reaction at 70 °C, the equilibrium conversion, being $48 \pm 1\%$, was reached after
126 10 min while the reaction at 35 °C reached an equilibrium conversion of $47 \pm 1\%$ after 30 min.

127 In order to obtain a higher yield of DAG, reactions were performed in the presence of petroleum
128 ether using a 1:1 mass ratio MAG/petroleum ether. Since petroleum ether is a nonpolar solvent, it
129 expelled the glycerol from the glyceride phase, thereby shifting the equilibrium towards DAG
130 formation (Fig. 1a). For solvent-assisted base-catalyzed reaction at 70 °C, the equilibrium conversion,
131 being $65 \pm 1\%$, was reached after 15 min; at 35 °C, an equilibrium conversion of $64 \pm 1\%$ was reached
132 after 50 min. When these data are compared to the analogue solvent-free reaction, the equilibrium
133 conversion increased by 17% when petroleum ether is used as a solvent.

134 Fig. 2 shows the concentrations of MAG, DAG and TAG in the reaction mixture for all reactions
135 performed with sodium methoxide as a catalyst. The highest amount of DAG was obtained at 70 °C
136 by using petroleum ether as solvent. However, when considering the TAG content, the highest
137 amount of TAG was also observed at these conditions.

138 *Solvent-free Acid-catalyzed Reactions*

139 Three acidic catalysts were used to convert MAG to DAG, p-toluenesulfonic acid, methanesulfonic
140 acid and sulfuric acid. Fig. 3 shows the amount of DAG versus time for all experiments. For the
141 reaction with p-toluenesulfonic acid at 70 °C, the equilibrium was achieved after 15 min while at 35
142 °C, equilibrium was not achieved within 60 min. However, when prolonging the reaction time,
143 equilibrium was reached after 150 min. The equilibrium conversions were $53 \pm 1\%$ at 35 °C and $56 \pm$

144 1% at 70 °C, both higher than the conversion which was achieved when using sodium methoxide as
145 catalyst. Using methanesulfonic acid, at 70 °C, equilibrium was reached within 15 min while at 35 °C,
146 it took 30 min to reach equilibrium. The equilibrium conversions were $74 \pm 3\%$ at 35°C and $73 \pm 1\%$ at
147 70 °C, which were higher than conversions reached when using sodium methoxide or p-
148 toluenesulfonic acid. With sulfuric acid as a catalyst equilibrium was reached after 15 min at 35 °C,
149 while at 70 °C, equilibrium was reached after 10 min. The equilibrium conversions were $73 \pm 2\%$ at 35
150 °C and $75 \pm 2\%$ at 70 °C which were in the same conversion range as when using methanesulfonic
151 acid. Fig. 4 summarizes the results for all alkali- and acid-catalyzed solvent-free reactions. It can be
152 seen that in alkali-catalyzed conditions, significantly more unwanted TAG are produced.
153 In order to determine the effects of catalyst concentration on reaction kinetics, the amount of
154 catalyst was reduced from 0.088 to 0.044 and 0.022 mol/mol. Lower concentration of catalyst
155 increases the time to reach equilibrium (Fig. 5). The amount of catalyst altered the equilibrium
156 conversion: $73 \pm 1\%$, $70 \pm 1\%$ and $65 \pm 2\%$ conversion were reached using 0.088, 0.044 and 0.022
157 mol/mol methanesulfonic acid, respectively. This phenomenon is discussed further on (see
158 Discussion - Comparison of the Reaction Equilibrium for the Acid-catalyzed Reactions).

159 *Solvent-assisted Acid-catalyzed Reactions*

160 The acidic catalysts used in solvent-free conditions were also tested in solvent-assisted reactions. Fig.
161 6 shows the amount of DAG versus time for all experiments. When p-toluenesulfonic acid was used
162 as catalyst, after 20 min of reaction at 70 °C, $82 \pm 2\%$ conversion was obtained. For reaction at 35°C
163 using the same catalyst, equilibrium was not reached within 60 min and even when reaction time
164 was extended to 300 min, equilibrium could not be reached. When comparing the results of the
165 solvent-assisted reactions with those of the solvent-free reactions, conversion was 26% higher when
166 petroleum ether was used. For reaction with methanesulfonic acid at 70 °C, $85 \pm 1\%$ conversion was
167 obtained after 10 min of reaction while at 35 °C, it took 30 min to reach $82 \pm 2\%$ conversion. Once
168 again, 8 to 12% increase in conversion was achieved when petroleum ether was added as solvent.
169 The last acid-catalyzed solvent-assisted reactions were performed using sulfuric acid as catalyst.

170 Equilibrium was achieved after 20 min at 70 °C, while at 35 °C, it took 50 min to achieve equilibrium.
171 The equilibrium conversion at 70 °C was $88 \pm 1\%$ while at 35 °C, the equilibrium conversion was $82 \pm$
172 2% . Fig. 7 summarizes the results for all solvent-assisted reactions. Once again, it can be seen that
173 more unwanted TAG are produced in base-catalyzed conditions. When comparing solvent-free and
174 solvent-assisted reactions (see Fig. 4 and Fig. 7), one can observe that the time to reach equilibrium
175 slightly increases when a solvent is added. However, conversion, and thus also the amount of DAG,
176 significantly increases in solvent-assisted conditions.

177 *Purification of the Reaction Mixture*

178 The crude reaction mixture contains TAG, DAG, MAG, glycerol, catalyst and traces of FFA. Phase
179 separation was observed in all reactions. The glycerol phase contained the polar compounds (glycerol
180 and catalyst), the glyceride phase contained the solvent (if added) and the glycerides. The literature
181 describes several methods to purify mixtures of different fatty acid compounds: liquid-liquid
182 extraction [23], molecular distillation [14,24] and column chromatography [11,20].

183 Two techniques were applied to obtain high-purity DAG: liquid-liquid extraction and column
184 chromatography. Simple liquid-liquid extraction with water removed glycerol and catalyst from the
185 crude mixture. The second step in this procedure aims to selectively extract the DAG from the
186 glyceride phase [23]. Several solvent mixtures were tested but gave unfavourable results in
187 separating the different glycerides. Hence, column chromatography was the most suitable technique.

188 Following the procedure mentioned earlier (see Experimental Procedures - Purification), about 70
189 samples were collected from the column. The TAG eluted first and were observed in fractions 8 to
190 24. Subsequently, DAG eluted (fractions 27 to 55) with the last part of eluent I (see Table 2),
191 however, the highest concentration of DAG was observed in the fractions containing eluent II.
192 Fractions 56 and higher contained the MAG and glycerol. The MAG were eluted with eluents III to V.
193 Glycerol was eluted with methanol.

194 The DAG were 96% pure (impurities mainly MAG) and were obtained at 92% recovery. Taking into
195 account 88% conversion (Fig. 7) this results in 81% overall yield of high-purity DAG. Thus, preparative
196 liquid chromatography or moving bed chromatography are promising possibilities for isolating DAG
197 on industrial scale.

198 Discussion

199 *Comparison of the Reaction Equilibrium for the Acid-catalyzed Reactions*

200 Fig. 8 shows the conversions for all three acidic catalysts when performing solvent-free or solvent-
201 assisted reactions at 35 or 70 °C. It can be seen that other degrees of conversion were reached when
202 different catalysts were used. This phenomenon can be explained by the fact that during reaction,
203 two phases were formed: a glycerol phase and a glyceride phase. A polar catalyst will go to the
204 glycerol phase, thereby increasing the polarity of this phase (Fig. 9). When the polarity of the glycerol
205 phase increases, more glycerol is attracted to this phase shifting the equilibrium toward DAG
206 formation (Fig. 1a). This hypothesis was confirmed by measuring the amount of glycerol and sulfur in
207 the glyceride phase after conducting solvent-free reactions at 35 °C with different acidic catalysts.

208 The reaction was carried out until equilibrium conversion was reached. Hence, reaction time
209 depended on the catalyst used, thus the reaction time was 180 min for p-toluenesulfonic acid, while
210 it was 60 min for methanesulfonic acid and sulfuric acid. Samples were taken after these reaction
211 times. The catalyst was not neutralized and the sample was centrifuged at 35 °C for 30 min. The
212 sulfur content and the glycerol content in the glyceride phase were measured, respectively by
213 means of XRF and GPC. From the results shown in Table 3, it can be seen that p-toluenesulfonic acid,
214 being the most apolar of the three catalysts, gave the highest glycerol content and the highest sulfur
215 content in the glyceride phase. Sulfuric acid, the most polar of the three catalysts, yielded no
216 detectable glycerol in the glyceride phase and had the lowest sulfur concentration in said layer. Thus,
217 GPC and XRF analyses confirmed that a more polar catalyst goes into the glycerol phase, leading to a
218 more polar glycerol phase, less glycerol in the glyceride phase, and thus to higher conversion. This
219 finding also clarifies why other equilibrium conversions are reached while using the same catalyst in

220 different concentrations (see Fig. 5). Higher concentration of catalyst cause the glycerol phase to be
 221 more polar and induces higher conversions.
 222 Extra experiments were conducted to determine the relationship between catalyst acidity and
 223 conversion. Reactions were performed at 70 °C for 60 min in solvent-free conditions using 0.088
 224 mol/mol acetic acid, phosphoric acid, or trichloroacetic acid as catalyst. In Fig. 10 the results of these
 225 reactions are compared with those of reactions with p-toluenesulfonic acid, methanesulfonic acid,
 226 and sulfuric acid (pKa values from reference 20). pKa values smaller than zero were needed to obtain
 227 > 50% conversion.

228 *Comparison of the Reaction Kinetics for the Acid-catalyzed Reactions*

229 In order to compare the kinetics of the different acid-catalyzed reactions, the amount of MAG was
 230 plotted versus reaction time and a nonlinear fit of these data was constructed. The general equation
 231 of these curves is shown in Eq. 3.

$$232 \quad n = n_0 + (n_{eq} - n_0) \cdot (1 - e^{-kt}) \quad [\text{Eq. 3}]$$

233 n = molar amount at time t [$\text{mol} \cdot 10^{-2}$]

234 n_0 = molar amount at time zero [$\text{mol} \cdot 10^{-2}$]

235 n_{eq} = equilibrium amount [$\text{mol} \cdot 10^{-2}$]

236 k = rate constant [min^{-1}]

237 t = time [min]

238 From Eq. 3, half-time values ($t_{1/2}$) can be calculated, which is the time for MAG to reach 50% of the
 239 original amount (n_0). Half-time values give good indications about reaction kinetics. The half-time
 240 value of MAG and the correlation coefficient of the fitted curves are given in Table 4. As expected,
 241 reactions at 70 °C had lower half-time values than those conducted at 35 °C. The solvent-assisted
 242 reactions were slower than the solvent-free reactions. This is due to a more efficient extraction of
 243 the catalyst to the glycerol phase in solvent-assisted conditions and dilution of the catalyst when
 244 adding petroleum ether.

245 *General conclusion*

246 Transesterification can be carried out both in base- and acid-catalyzed modes. However, unwanted
247 formation of TAG is suppressed in acid-catalyzed conditions. In order to reach high equilibrium
248 amounts of DAG, the pK_a value of the acidic catalyst should be < 0 . The polarity of the catalyst also
249 plays an important role: more polar catalysts move to the polar glycerol phase during reaction,
250 thereby increasing the polarity of the glycerol phase and shifting the equilibrium in favour of DAG
251 production. Consequently, higher conversions are achieved with more polar catalysts. Although
252 acceptable conversion was achieved in solvent-free reactions, conversions can be increased by using
253 a solvent. The solvent petroleum ether can easily be distilled and reused because of its low boiling
254 point. Solvent-assisted reactions have slower kinetics, but equilibrium is still reached after 20 min.
255 Maximum conversion (88%) was achieved in sulfuric acid-catalyzed, solvent-assisted reaction at 70
256 °C. Purification (column chromatography) achieved 92% recovery of 96% pure DAG. An overall yield
257 of 81% was achieved. The methods presented in this work are suitable for large-scale production of
258 high-purity DAG. Although higher conversions are reached when working with petroleum ether as
259 solvent, this method is less environmentally benign. Moreover, the overall cost of the process will
260 increase when a solvent is used. Thus, both economic and environmental parameters will need to be
261 evaluated in order to choose the right process for large-scale production.

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- 320 Fig. 1 (a) transesterification of 2 MAG molecules, (b) transesterification of a MAG and a DAG
321 molecule, (c) transesterification of a MAG and a TAG molecule
- 322 Fig. 2 Base-catalyzed transesterification of MAG using sodium methoxide catalyst, concentration of
323 MAG, DAG and TAG in the glyceride phase after 60 min of reaction (for solvent-assisted reactions,
324 petroleum ether was evaporated before GPC measurement)
- 325 Fig. 3 DAG concentration versus time for solvent-free transesterification of MAG at (a) 35 °C and (b)
326 70 °C (PTSA=p-toluenesulfonic acid, MSA=methanesulfonic acid, SFA=sulfuric acid)
- 327 Fig. 4 Overview of the results for all solvent-free reactions (SMO=sodium methoxide, PTSA=p-
328 toluenesulfonic acid, MSA=methanesulfonic acid, SFA=sulfuric acid), * for PTSA at 35 °C the
329 equilibrium time was 300 min
- 330 Fig. 5 Solvent-free, acid-catalyzed transesterification of MAG using different amounts of catalyst
331 (methanesulfonic acid, 70°C)
- 332 Fig. 6 DAG concentration versus time for solvent-assisted transesterification of MAG at (a) 35 °C and
333 (b) 70 °C (PTSA=p-toluenesulfonic acid, MSA=methanesulfonic acid, SFA=sulfuric acid)
- 334 Fig. 7 Overview of the results for all solvent-assisted reactions (SMO=sodium methoxide, PTSA=p-
335 toluenesulfonic acid, MSA=methanesulfonic acid, SFA=sulfuric acid), * for PTSA at 35 °C reaction time
336 was 300 min (equilibrium could not be reached)
- 337 Fig. 8 Conversion for synthesis of DAG from MAG at 70°C and 35°C in solvent-assisted (SA) and
338 solvent-free (SF) conditions using different acidic catalysts (PTSA = p-toluenesulfonic acid, MSA =
339 methanesulfonic acid, SFA = sulfuric acid), standard reaction time of 60 min (prolonged to 180 min
340 for PTSA-SF in order to reached equilibrium, prolonged to 300 min for PTSA-SA without reaching
341 equilibrium)
- 342 Fig. 9 Schematic representation of catalyst movement to glycerol layer for solvent-free and solvent-
343 assisted reactions with (a) p-toluenesulfonic acid, (b) methanesulfonic acid and (c) sulfuric acid. Line
344 in the middle represents the phase separation.
- 345 Fig. 10 Solvent-free acid-catalyzed transesterification of MAG using different acidic catalysts (60 min,
346 70°C), pKa versus conversion (pKa values from reference 22)
- 347

348 Table 1 Fatty acid profile of starting material (MAG from Oleon)

Fatty acid	% (w/w)
C16:0	7.9
C18:1t	3.1
C18:1c	35.2
C18:2	53.5
C18:3	0.3

349

350 Table 2 Solvent mixtures applied for column elution with reduced solvent use

Volume (mL)	Solvents	Mixture (v/v)
1200	Petroleum ether/ethyl acetate/acetic acid (I)	90:10:1
300	Petroleum ether/ethyl acetate/acetic acid (II)	80:20:1
600	Petroleum ether/ethyl acetate/acetic acid (III)	70:30:0.7
300	Petroleum ether/ethyl acetate/acetic acid (IV)	50:50:1
100	Ethyl acetate (V)	100
600	Methanol (VI)	100

351

352 Table 3 Conversion and concentration of sulfur and glycerol in the glyceride layer after solvent-free
 353 reaction at 35 °C using different acidic catalysts (PTSA = p-toluenesulfonic acid, MSA =
 354 methanesulfonic acid, SFA = sulfuric acid), samples were taken after equilibrium concentration was
 355 reached (180 min for PTSA, 60 min for MSA and SFA)

	Concentration in glyceride phase		Conversion [%]
	Sulfur [ppm]	Glycerol [% w/w]	
PTSA	4410	6.8	55
MSA	1795	1.4	73
SFA	65	0.0	77

356

357 Table 4 Half time values [min] and correlation coefficients after nonlinear fit of amount of MAG
 358 (PTSA = p-toluenesulfonic acid, MSA = methanesulfonic acid, SFA = sulfuric acid)

Catalyst	35 °C				70 °C			
	Solvent-free		Solvent-assisted		Solvent-free		Solvent-assisted	
	$t_{1/2}$	R^2	$t_{1/2}$	R^2	$t_{1/2}$	R^2	$t_{1/2}$	R^2
PTSA	30.00	0.9977	47.40	0.9904	3.13	0.9919	5.03	0.9926
MSA	1.47	0.9587	3.06	0.9731	0.50	0.9965	0.56	0.9964
SFA	0.99	0.9654	3.03	0.9603	0.45	0.9892	0.46	0.9883

359

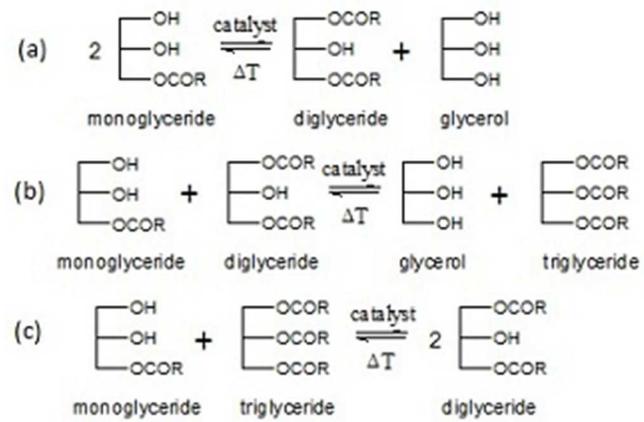


Fig. 1 (a) transesterification of 2 MAG molecules, (b) transesterification of a MAG and a DAG molecule, (c) transesterification of a MAG and a TAG molecule
85x56mm (96 x 96 DPI)

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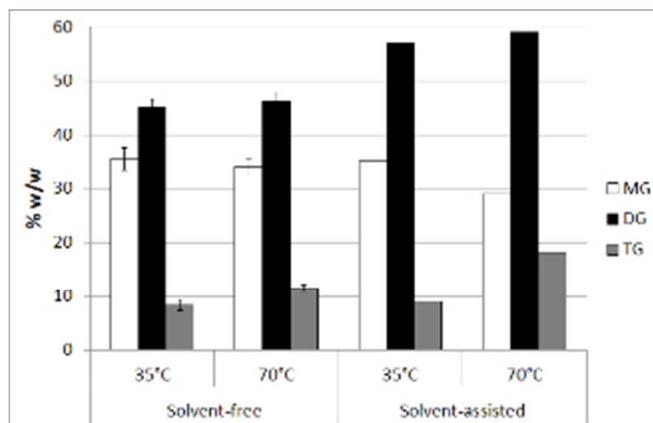


Fig. 2 Base-catalyzed transesterification of MAG using sodium methoxide catalyst, concentration of MAG, DAG and TAG in the glyceride phase after 60 min of reaction (for solvent-assisted reactions, petroleum ether was evaporated before GPC measurement)
87x55mm (96 x 96 DPI)

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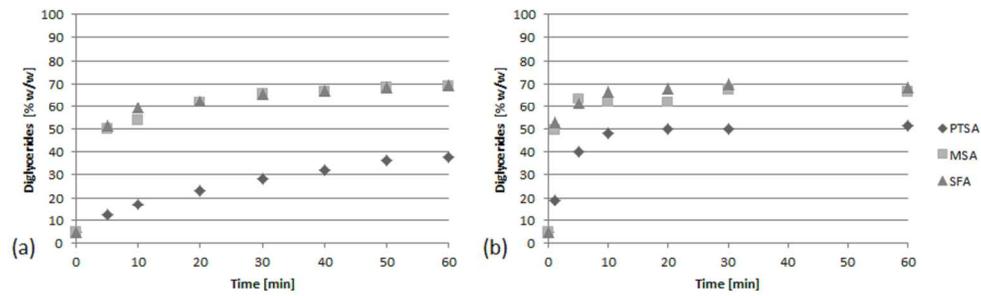


Fig. 3 DAG concentration versus time for solvent-free transesterification of MAG at (a) 35 °C and (b) 70 °C (PTSA=p-toluenesulfonic acid, MSA=methanesulfonic acid, SFA=sulfuric acid)
485x152mm (96 x 96 DPI)

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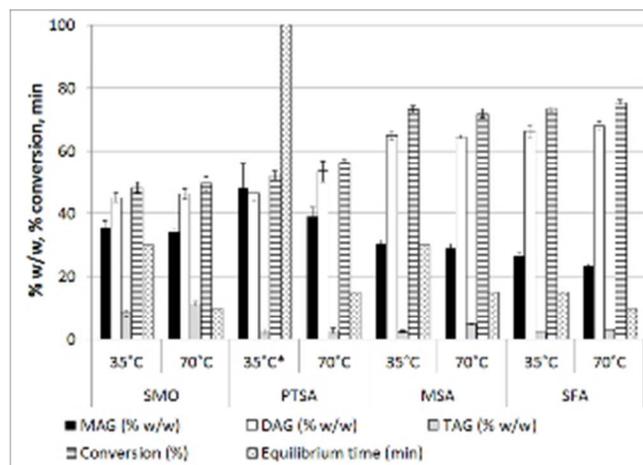


Fig. 4 Overview of the results for all solvent-free reactions (SMO=sodium methoxide, PTSA=p-toluenesulfonic acid, MSA=methanesulfonic acid, SFA=sulfuric acid), * for PTSA at 35 °C the equilibrium time was 300 min
85x61mm (96 x 96 DPI)

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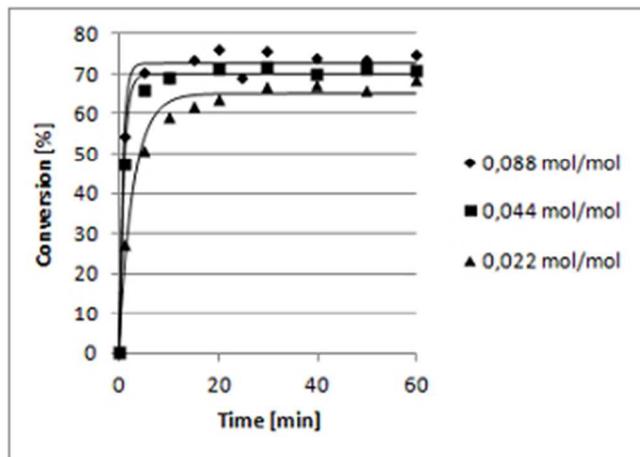


Fig. 5 Solvent-free, acid-catalyzed transesterification of MAG using different amounts of catalyst (methanesulfonic acid, 70°C)
85x61mm (96 x 96 DPI)

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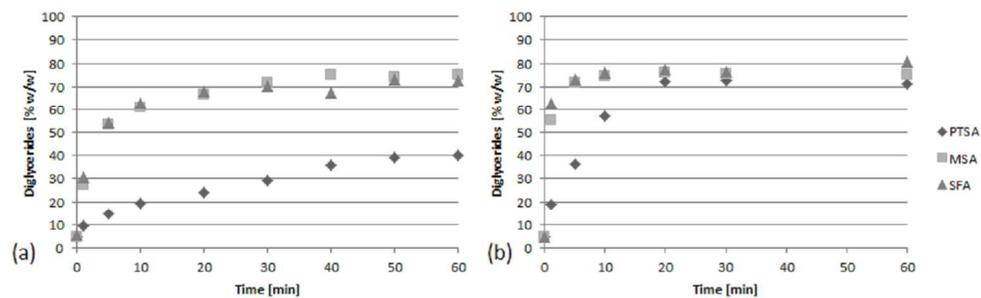


Fig. 6 DAG concentration versus time for solvent-assisted transesterification of MAG at (a) 35 °C and (b) 70 °C (PTSA=p-toluenesulfonic acid, MSA=methanesulfonic acid, SFA=sulfuric acid)
361x114mm (96 x 96 DPI)

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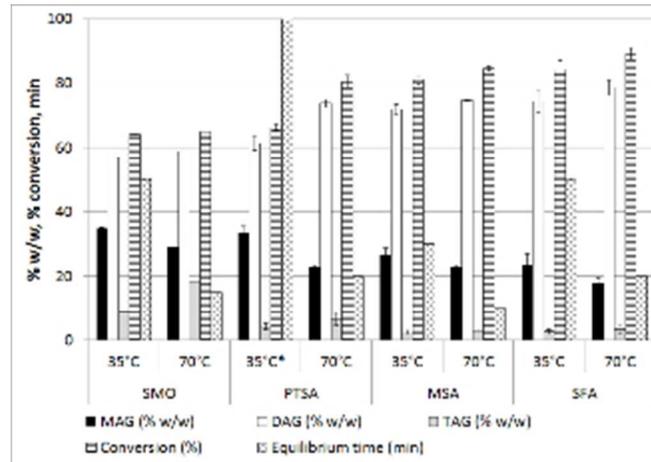


Fig. 7 Overview of the results for all solvent-assisted reactions (SMO=sodium methoxide, PTSA=p-toluenesulfonic acid, MSA=methanesulfonic acid, SFA=sulfuric acid), * for PTSA at 35 °C reaction time was 300 min (equilibrium could not be reached)
87x61mm (96 x 96 DPI)

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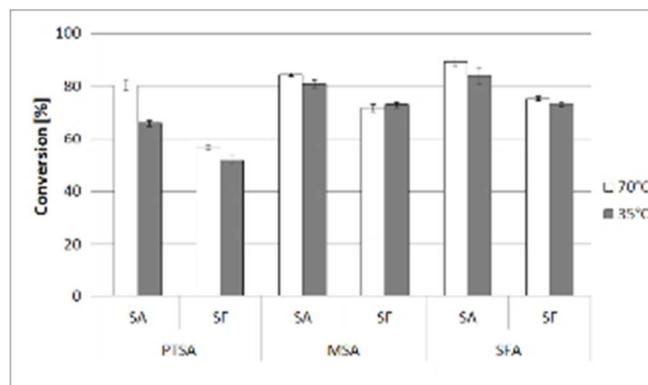


Fig. 8 Conversion for synthesis of DAG from MAG at 70°C and 35°C in solvent-assisted (SA) and solvent-free (SF) conditions using different acidic catalysts (PTSA = p-toluenesulfonic acid, MSA = methanesulfonic acid, SFA = sulfuric acid), standard reaction time of 60 min (prolonged to 180 min for PTSA-SF in order to reached equilibrium, prolonged to 300 min for PTSA-SA without reaching equilibrium)
86x50mm (96 x 96 DPI)

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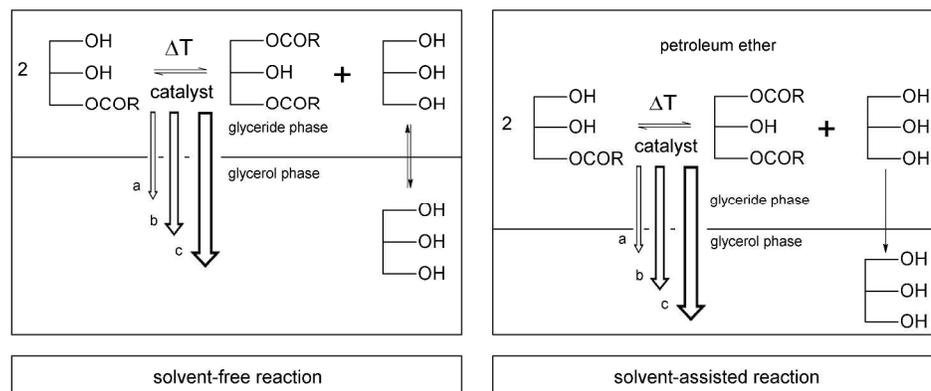


Fig. 9 Schematic representation of catalyst movement to glycerol layer for solvent-free and solvent-assisted reactions with (a) p-toluenesulfonic acid, (b) methanesulfonic acid and (c) sulfuric acid. Line in the middle represents the phase separation.

341x133mm (300 x 300 DPI)

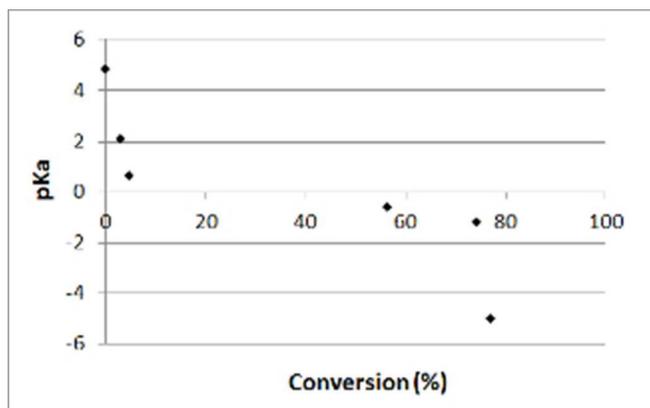


Fig. 10 Solvent-free acid-catalyzed transesterification of MAG using different acidic catalysts (60 min, 70°C), pKa versus conversion (pKa values from reference 22)
86x53mm (96 x 96 DPI)

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