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2 Thermochemolysis using TMAAc and TMAH reagents as means to
3 differentiate between free acids and esters

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9 *Abstract*

10 The reactivity of aromatic and aliphatic esters in on-line thermochemolysis in the presence of
11 methylation reagents was studied using model compounds. Guaiacyl and 2-nonyl palmitates
12 were synthesised, representing aromatic and aliphatic ester bonds, respectively. These model
13 compounds were analysed by on-line thermochemolysis using tetramethylammonium acetate
14 (TMAAc) and tetramethylammonium hydroxide (TMAH) in order to differentiate free acids
15 from esterified acids. The released palmitic acid with TMAH for both model compounds was
16 close to the theoretical palmitic acid content in the model compounds, even if part of the
17 aliphatic ester had not reacted with TMAH. The free palmitic acid content by TMAAc was
18 one third of the theoretical value for aromatic ester and only a few percentages for aliphatic
19 ester. The result indicated that the less basic reagent is able to hydrolyse the aromatic ester
20 linkage to some extent, whereas the aliphatic ester remains intact. Thus, differentiation of free
21 acids by TMAAc from the esterified acids cannot be reliably performed from matrices
22 containing aromatic esters. It was found that transesterification due to the use of methanol as
23 a solvent with TMAAc is an insignificant reaction in on-line thermochemolysis.

24 **Keywords:** Thermochemolysis, tetramethylammonium hydroxide, tetramethylammonium
25 acetate, ester bond, free acids, Py-GC/MS

26 **1. Introduction**

27 The on-line derivatisation method “thermally assisted hydrolysis and methylation” in the
28 presence of the reagent tetramethylammonium hydroxide (TMAH) was developed by
29 Challinor [1]. The method, also called thermochemolysis, has been widely used for the
30 characterization of a variety of natural materials and synthetic polymers reviewed by
31 Challinor [2] and Shadkani [3]. TMAH is a strong base and it cleaves ester and ether bonds
32 extensively, forming more volatile methyl ester and ether derivatives [4]. TMAH is supposed
33 to react also with the free acids present in the sample. Therefore, it can be used to determine
34 the total fatty acid content of the sample [5-7]. Another thermochemolysis reagent,
35 tetramethylammonium acetate (TMAAc), is more selective. It reacts only with free acid and
36 alcohol groups. It has been used together with TMAH to differentiate between free and
37 esterified fatty acids in wood extractives [6]. Tetraethylammonium acetate (TEAAc) is
38 another reagent that also has been applied to give information of the free acids present in the
39 sample. Instead of methylation, ethylated products are formed. The advantage of TEAAc is
40 that it can be used to detect methyl esters originally present in the samples separately from
41 the free acids [8]. TMAAc and TEAAc have mainly been used together with more basic
42 thermochemolysis reagents like TMAH and tetrabutylammonium hydroxide (TBAH) for the
43 analysis of lipids from natural materials including humic and humin substances [9-10] as well
44 as earlier mentioned wood extractives [6, 11]. In addition, aquatic natural organic matter,
45 which contains mainly phenolic structures, has been studied with different thermochemolysis
46 reagents [12]. In that study, methylation reaction with TMAAc (in methanol) was explained
47 to occur partly via transesterification. In addition to free functional groups, part of the ester
48 bonds present in the natural organic matter was supposed to react with the methoxide ion to
49 form methyl esters. The methoxide ion was suggested to derive from the methanol used as
50 TMAAc solvent. The measured free acid content would thus be higher than the actual fatty

51 acid content present in the sample. The measurement was done using off-line
52 thermochemolysis. It is expected that transesterification does not take place in on-line
53 thermochemolysis. Triglycerides were analysed also with TMAAc and other more basic
54 reagents [7]. Quite high proportion of acids was in free form. Therefore, it was concluded that
55 part of the ester bond might be cleaved at the high temperature thermochemolysis with
56 TMAAc. Though TMAAc and TEAAc are used to determine free acid in different type of
57 natural polymers containing both aromatic and aliphatic ester bonds, it has not been reported
58 in literature whether TMAAc and TEAAc react differently with the two types of esters.
59 Before using reagents with different alkalinity in the analysis of modified biopolymers
60 containing both aliphatic and aromatic ester linkages, two model compounds, guaiacyl
61 palmitate and 2-nonyl palmitate were synthesised, representing the aromatic ester bond
62 between phenol and fatty acid and aliphatic ester bond between aliphatic alcohol and fatty
63 acid, respectively. These model compounds were analysed using TMAH and TMAAc in
64 order to understand the behaviour of the reagents in the analysis of ester bonds and free acids.
65 Even if it has been claimed that transesterification probably does not take place in on-line
66 thermochemolysis this possibility was studied in the present study, and it was found to be
67 negligible, as expected.

68 **2. Material and methods**

69 *2.1. The general procedure for model compound*

70 All materials used for the synthesis were commercial and used as such unless otherwise
71 noted. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance III 500 MHz NMR
72 spectrometer and the chemical shifts were calibrated to the residual proton and carbon
73 resonance of the deuterated solvent.

74 Guaiacyl palmitate: palmitic acid (7.43 g, 29.0 mmol), quaiacol (3.42 g, 27.6 mmol) and 4-
75 dimethylaminopyridine (0.67 g, 5.52 mmol) were dissolved in dichloromethane (100 ml) and
76 cooled to 0°C, followed by addition of dicyclohexanecarbodiimide (6.83 g, 33.1 mmol)
77 portion wise in a dichloromethane solution. The reaction was left to reach room temperature
78 overnight and was then filtered to remove solids. The filtrate was evaporated and the crude
79 product was purified by flash chromatography from heptane increasing polarity to
80 EtOAc:Heptane 5:95 to give product as a white solid. Yield was 8.32 g (83%).

81 ¹H NMR (500.1 MHz, CDCl₃): δ 0.88 (t, *J* = 6.70 Hz, 3H, -CH₃), 1.26-1.37 (m, 22H, -CH₂),
82 1.43 (t, *J* = 7.11 Hz, 2H, -CH₂), 1.77 (t, *J* = 7.46 Hz, 2H, -CH₂), 2.58 (t, *J* = 7.46 Hz, 2H, -
83 CH₂), 3.82 (s, 3H, -OCH₃), 6.93-7.21 (m, 4H, -ArH). ¹³C NMR (125.8 MHz, CDCl₃): δ 14.11
84 (-CH₃), 22.67 (-CH₂), 25.04 (-CH₂), 29.06 (-CH₂), 29.28 (-CH₂), 29.35 (-CH₂), 29.50 (-CH₂),
85 29.61 (-CH₂), 29.65-29.68 (5C, -CH₂), 31.91 (-CH₂), 34.05 (-CH₂), 55.77 (-OCH₃), 112.35
86 (ArCH), 120.72 (ArCH), 122.82 (ArCH), 126.71 (ArCH), 139.84 (ArC), 151.14 (ArC),
87 171.89 (-CO).

88 2-Nonyl palmitate: Palmitic acid (7.04 g, 27.4 mmol), 2-nonanol (3.77 g, 26.1 mmol) and 4-
89 dimethylaminopyridine (0.64 g, 5.23 mmol) were dissolved in dichloromethane (100 ml) and
90 cooled to 0°C, followed by addition of dicyclohexanecarbodiimide (6.47 g, 31.4 mmol)
91 portion wise from a dichloromethane solution. The reaction was left to reach room
92 temperature overnight and was then filtered to remove solids. Filtrate was evaporated and the
93 crude product was purified by flash chromatography from heptane increasing polarity to
94 EtOAc:Heptane 2:98 to give product as a clear oil. Yield 7.17 g (71.7 %).

95 Yield of product was 7.17 g (72%).

96 ¹H NMR (500 MHz, CDCl₃): δ 0.88 (t, *J* = 6.93 Hz, 6H, -CH₃), 1.19 (d, *J* = 6.25 Hz, 3H,
97 CH₃), 1.25-1.34 (m, 34H, CH₂), 1.42-1.63 (m, 4H, -CH₂), 2.26 (t, *J* = 7.53 Hz, 2H, -CH₂),

98 4.89 (hex, $J = 6.32$ Hz, 1H, -CH). ^{13}C NMR (126 MHz, CDCl_3): δ 14.07 (- CH_3), 14.11 (-
99 CH_3), 20.02 (- CH_3), 22.64 (- CH_2), 22.69 (- CH_2), 25.12 (- CH_2), 25.42 (- CH_2), 29.15 (- CH_2),
100 29.21 (- CH_2), 29.29 (- CH_2), 29.36 (- CH_2), 29.41 (- CH_2), 29.48 (- CH_2), 29.60 (- CH_2), 29.65-
101 29.69 (5C, - CH_2), 31.78 (- CH_2), 31.92 (- CH_2), 34.77 (- CH_2), 35.96 (- CH_2), 70.71 (-CH),
102 173.58 (-CO).

103 2.2. Thermochemolysis

104 Thermochemolysis with TMAH (10% in aqueous solution, Merck) and TMAAc (15% in
105 aqueous solution, TCI) was performed using a platinum foil pulse pyrolyzer (Pyrolab2000@
106 from Pyrolab, Sweden) connected to a gas chromatograph mass spectrometer (Varian 3800
107 GC-Varian Saturn 2000 MS). A fused silica capillary column (J&W, DB-1701, 30 m x 0.25
108 mm, film thickness 1 μm) was used for the compound separation. 2 to 4 μl of the sample
109 solution (model compounds diluted to methanol or dichloromethane) at concentration about
110 0.5 mg/ml was pipetted on the filament together with internal standard (heneicosanoic acid,
111 Fluka purum $\geq 98\%$) and the reagent. The mixture was inserted to the pyrolyzer maintained at
112 175°C and the filament was heated to temperatures between 280 and 600°C depending on the
113 reagent used. Pyrolysis time was 2 s. After pyrolysis, the degradation products were led into
114 the capillary column for separation using helium as carrier gas at flow rate 1.0 ml min^{-1} . The
115 column temperature was programmed from 80°C (2 min) to 160°C at 8°C min^{-1} and
116 extended from 160°C to 280°C at 5°C min^{-1} . The final temperature was held for 5 min. The
117 mass spectrometer was operated in EI mode (70 eV) using mass range of m/z 46-650. Acid
118 content was calculated using internal standard calibration.

119

120 **3. Results and discussions**

121 *3.1. Thermochemolysis conditions*

122 Two model compounds guaiacyl palmitate and 2-nonyl palmitate, which contained both
123 aromatic and aliphatic ester bonds, were synthesised. Structures of model compounds are
124 shown in Fig. 1. These model compounds were analysed using on-line thermochemolysis
125 with two methylation reagents TMAH and TMAAc. On-line thermochemolysis was
126 performed in a pyrolysis unit. Therefore, short reaction time of 2 seconds was favoured. Both
127 methylation reagents were used as aqueous solutions. Based on literature, thermochemolysis
128 at pyrolysis temperature of 600°C has given highest recovery for the fatty acids [7]. Our
129 experience was that 400°C and 600°C gave basically the same yield of fatty acid. However, at
130 high temperatures such as 600°C, better yield for the aromatic compounds were obtained (not
131 reported). Therefore, TMAH thermochemolysis was performed at 600°C, whereas lower
132 temperature was used for TMAAc thermochemolysis to avoid thermal degradation of the
133 ester bond. Both model compounds as well as the internal standard were diluted to methanol
134 and dichloromethane before transferring on the filament. The reason to use two solvents with
135 different structure was to understand the effect of the solvents on the methylation reaction i.e.
136 transesterification. If transesterification of the ester groups in the sample take place via
137 methoxide ion formation, higher proportion of methylated products would be formed when
138 methanol is used as a solvent in thermochemolysis by TMAAc. It was proposed that
139 transesterification does not take place when dichloromethane is used as a solvent. The
140 solutions were prepared for the model compounds in order to be able to transfer a suitable
141 amount of sample on the filament and avoid overload of the detector. In TMAAc
142 thermochemolysis, the reagent was mixed with the sample in two different ways: before or
143 after addition of the sample on the filament. The concentration of TMAAc in the mixture was
144 3%. There was no difference in the results depending on how the reagent was mixed with the

145 sample. Therefore, the results are presented as average. TMAH was always added after
146 sample addition directly on the filament. All measurements were done at least triplicate. The
147 released palmitic acid content was calculated for the model compounds using internal
148 standard calibration.

149

150 *3.2. Thermochemolysis by TMAH and TMAAc*

151 The expected reactions taking place in TMAH thermochemolysis of guaiacyl and 2-nonyl
152 palmitate are shown in Fig. 2. It was expected that TMAAc does not react with ester bonds.
153 Total ion gas chromatograms in Fig. 3 and Fig. 4 show the methylated products formed from
154 the guaiacyl palmitate and 2-nonyl palmitate in thermochemolysis with TMAH and TMAAc.
155 In the presence of TMAH, guaiacyl palmitate was hydrolysed and palmitic acid methyl ester
156 and dimethoxybenzene were formed as methylation products (Fig. 3). The peak intensities of
157 dimethoxybenzene and palmitic acid methyl ester were surprisingly high in comparison to
158 internal standard intensity when TMAAc was used, as TMAAc is not supposed to hydrolyse
159 esters [6]. The result indicates that part of the aromatic ester was hydrolysed in the
160 thermochemolysis by TMAAc. In addition to methylated products, also nonmethylated
161 guaiacol was detected probably as a thermal degradation product of guaiacyl palmitate.
162 Nonmethylated palmitic acid was not detected, probably because the elution of fatty acids
163 without derivatisation is poor.

164 Methyl palmitate was the only methylated product from 2-nonyl palmitate in the presence of
165 TMAH (Fig. 4). Small peak of 2-nonanol was detected, but not 2-nonyl methyl ether. It has
166 earlier been reported that all alcohol groups are not fully methylated [1]. Also small amount
167 of the original ester 2-nonyl palmitate was found, indicating incomplete reaction of aliphatic

168 esters in the presence of TMAH. In the thermochemolysis of 2-nonyl palmitate with TMAAc,
169 only a small peak of methyl palmitate was found, indicating that only the free acid is reacted
170 with TMAAc, as expected. The peak with highest abundance was the unreacted ester. In
171 addition, a small amount of non-methylated 2-nonanol was detected. Our results regarding
172 the thermochemolysis of aliphatic esters by TMAAc were consistent with the results reported
173 in literature [6].

174 The amount of released palmitic acid was calculated for the TMAH and TMAAc
175 thermochemolysis experiments. The palmitic acid content determined by TMAH was
176 compared with the theoretical content. The results are shown in Fig. 5 and Fig. 6. The
177 measured total palmitic acid content for the guaiacyl palmitate was close to the theoretical
178 value (Fig. 5). When methanol was used as solvent, the average palmitic acid content was
179 closer to the theoretical value than in the case of dichloromethane as solvent. However, the
180 standard deviation was higher when methanol was used. The released palmitic acid content
181 from the guaiacyl palmitate with TMAAc was about one third of the theoretical total content.
182 Again higher average palmitic acid content was measured when methanol was used as
183 solvent as compared to dichloromethane. However, the methylation of palmitic acid cannot
184 explain to form via transesterification, because palmitic acid content was equal with both
185 solvents. The measured free palmitic acid content was surprisingly high, as any unreacted
186 palmitic acid after the synthesis was supposed to be removed during the purification, further
187 verified by NMR analysis of the product. The origin of the measured high free palmitic acid
188 content is thus concluded to be partial hydrolysis of the aromatic ester bond in the
189 thermochemolysis by TMAAc.

190 The palmitic acid content determined with TMAH from the 2-nonyl palmitate was slightly
191 lower than the theoretical value, as part of the 2-nonyl palmitate had not reacted with TMAH

192 (Fig. 6). A small peak of the original ester is seen in the total ion gas chromatogram,
193 verifying the conclusion (Fig. 4). A small amount of palmitic acid was measured in the
194 presence of TMAAc from the 2-nonyl palmitate. The palmitic acid content measured from 2-
195 nonyl palmitate was much lower in comparison to the palmitic acid content measured from
196 guaiacyl palmitate. In the case of 2-nonyl palmitate, TMAAc seems to react only with the
197 free acid group, not with the ester group, as expected. The result proved that the aliphatic
198 ester bond is more stable against less basic reagents such as TMAAc in comparison to the
199 aromatic ester bond. Also in this case, the palmitic acid content was slightly higher when the
200 samples were diluted in methanol rather than dichloromethane. However, the standard
201 deviation was large when methanol was used. Due to the similar values obtained with both
202 solvents it was proposed that transesterification due to the use of methanol as solvent with
203 TMAAc is an insignificant reaction in on-line thermochemolysis.

204 The influence of pyrolysis temperature on palmitic acid yield was studied in the case of 2-
205 nonyl palmitate, because part of the ester was not hydrolysed and methylated in the presence
206 of TMAH. However, there were no differences in palmitic acid content at temperatures of
207 600, 700 and 800°C, indicating that hydrolysis and methylation was independent on the
208 temperature changes (Fig. 7). The influences of reaction time before pyrolysis and pyrolysis
209 time on palmitic acid yield were not studied.

210 **4. Conclusions**

211 TMAH has been used for the determination of total fatty acids content. In this study, TMAH
212 was used to determine total palmitic acid content from model compounds that represented
213 both aliphatic and aromatic ester bonds between alcohol and fatty acid. Palmitic acid was
214 hydrolysed and methylated totally from the aromatic ester, whereas part of the aliphatic ester

215 remained stable. TMAAc has earlier been used to differentiate between free and esterified
216 acids in wood extractives. The fatty acids in wood extractives are esterified with cyclic and
217 aliphatic alcohols. The results obtained in this study showed that the aromatic ester bond is
218 partly hydrolysed and methylated in the presence of TMAAc, whereas the aliphatic ester
219 bond is stable against TMAAc. It means that TMAAc can be used to distinguish between free
220 acids and aliphatic esters in matrices such as wood extractives, containing only aliphatic
221 alcohols. However, it cannot be used to determine free acids from samples, which contain either
222 aromatic and aliphatic ester linkages, or only aromatic ester linkages. Transesterification in
223 thermochemolysis in the presence of methanol has been suggested to increase the yield of
224 methylated products. However, it was now shown that in on-line thermochemolysis, the
225 transesterification reaction is insignificant.

226

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Figure legends

Fig. 1. Model compounds

Fig. 2. Expected products formed in TMAH thermochemolysis of guaiacyl palmitate (above) and 2-nonyl palmitate (below)

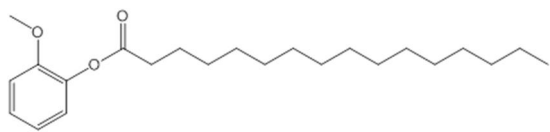
Fig. 3. Products formed from guaiacyl palmitate (in dichloromethane) by TMAH thermochemolysis (above) and TMAAc thermochemolysis (below).

Fig. 4. Products formed from 2-nonylpalmitate (in dichloromethane) by TMAH thermochemolysis (above) and TMAAc thermochemolysis (below)

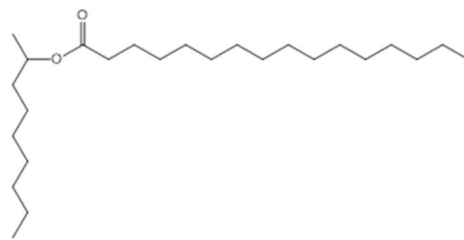
Fig. 5. Amount of palmitic acid released from guaiacyl palmitate by TMAH and TMAAc.

Fig. 6. Amount of palmitic acid released from 2-nonyl palmitate by TMAH and TMAAc

Fig. 7. Effect of temperature on palmitic acid yield from 2-nonyl palmitate (in dichloromethane) by TMAAc. One measurement was done at 800°C.



Guaiacyl palmitate



2-nonyl palmitate

Fig. 1. Model compounds

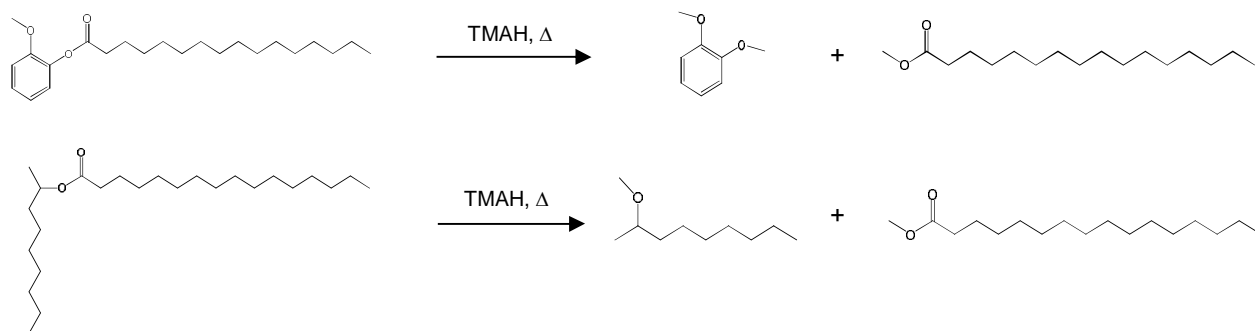


Fig. 2. Expected products formed in TMAH thermochemolysis of guaiacyl palmitate (above) and 2-nonyl palmitate (below)

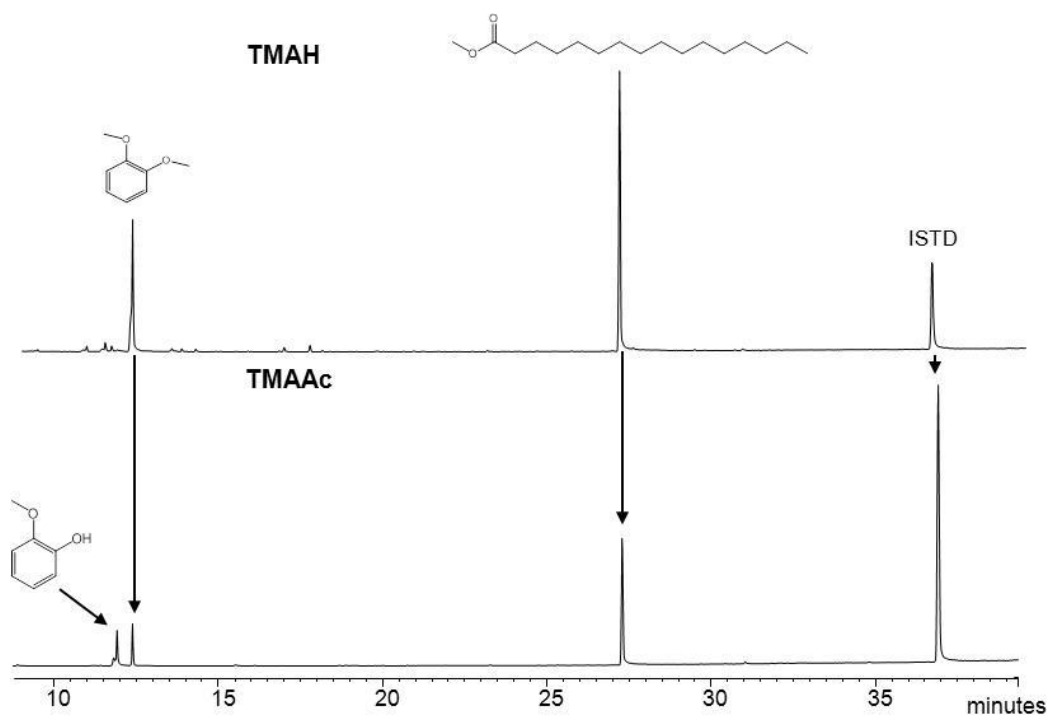


Fig. 3. Products formed from guaiacyl palmitate (in dichloromethane) by TMAH thermochemolysis (above) and TMAAc thermochemolysis (below).

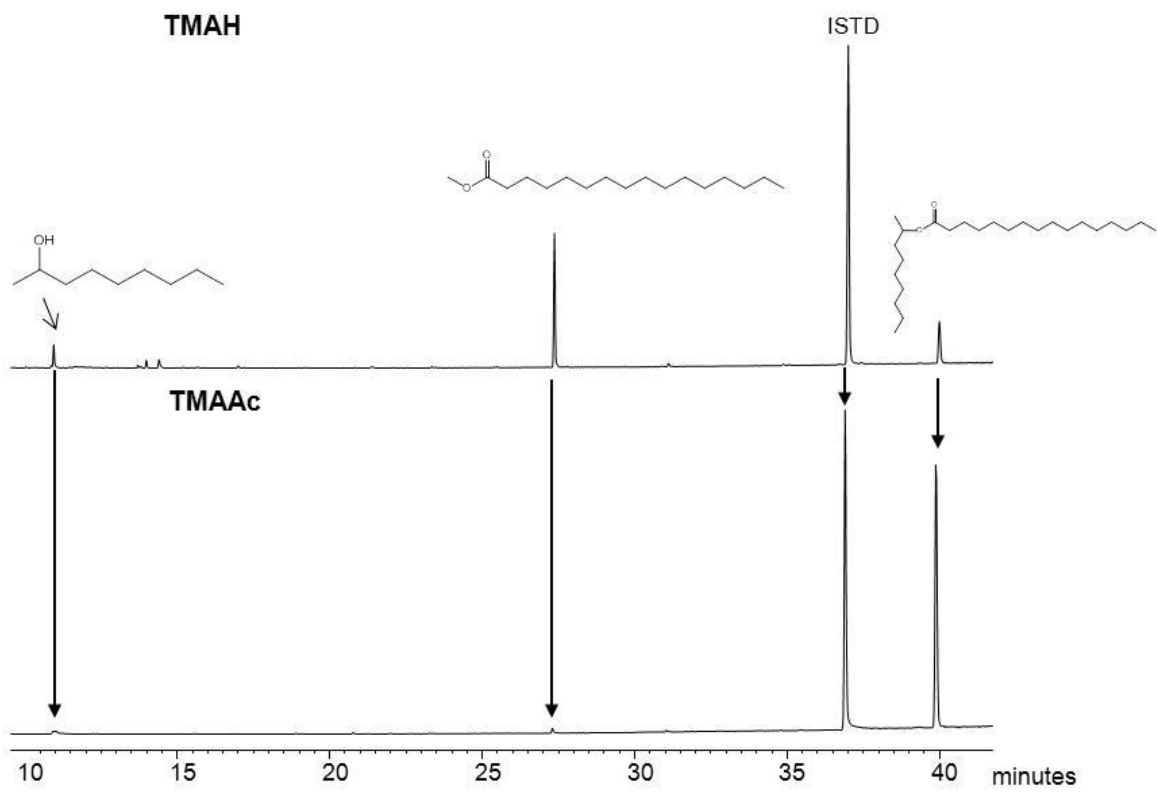


Fig. 4. Products formed from 2-nonyl palmitate (in dichloromethane) by TMAH thermochemolysis (above) and TMAAc thermochemolysis (below)

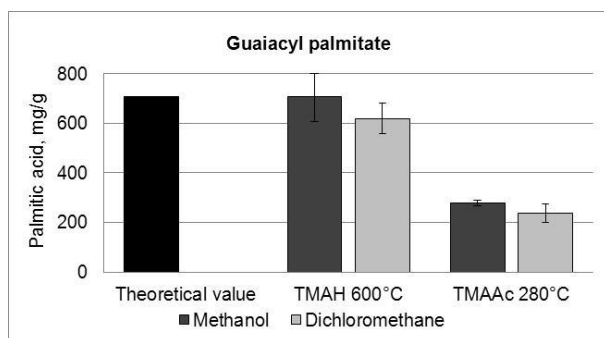


Fig 5. Amount of palmitic acid released from guaiacyl palmitate by TMAH and TMAAc.

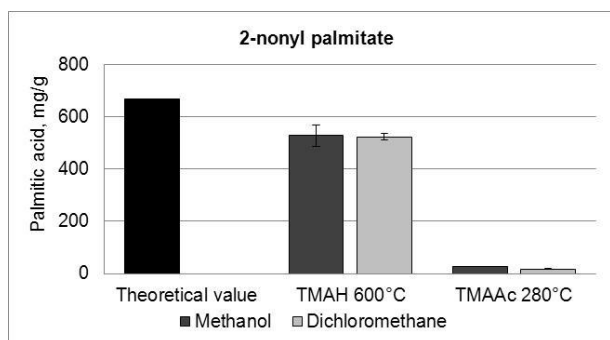


Fig 6. Amount of palmitic acid released from 2-nonyl palmitate by TMAH and TMAAc

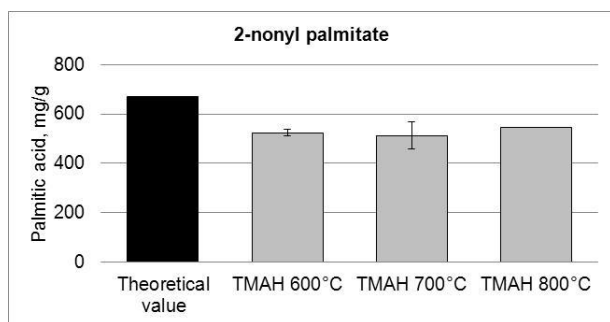


Fig. 7. Effect of temperature on palmitic acid yield from 2-nonyl palmitate (in dichloromethane) by TMAAc.