Molecular changes in egg tempera paint dosimeters as tools to monitor the museum environment
van den Brink, O.F.

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5. Cholesterol oxidation products in light-aged egg tempera

Cholesterol constitutes about 5% of the lipid fraction of eggs. The question whether cholesterol is a stable compound in egg tempera dosimeters is addressed in this chapter. Earlier, we have indicated in Chapter 2 that cholesterol oxidises in egg tempera paint under the conditions of light exposure, which makes it an important marker for dosimetry. Further evidence is provided here to support the assignments of characteristic mass peaks to specific cholesterol oxidation products (COPs). The idea that cholesterol might be a marker for egg tempera paint [1] is challenged.

5.1 Introduction

The oxidation of cholesterol in the human body has been studied extensively in relation to atherosclerosis and cancer. A great variety of tens of COPs has been identified in an even greater number of different materials [2, 3]. These include compounds that are formed in the very early stage of oxidation (cholesterol hydroperoxides) as well as more stable oxidation products. A strongly preferred position for the autoxidation of cholesterol is the 7-position (see Scheme 1 for the numbering of the carbon atoms in cholesterol). The initiation of the autoxidation reaction involves the formation of a carbon-centred allylic C-7 radical, which reacts with molecular oxygen and subsequently abstracts a hydrogen, leading to the formation of 7-hydroperoxycholesterol. This compound can then react to 3-hydroxycholesterol-5-en-7-one and cholest-5-ene-3,7-diol, which are known as the major autoxidation products of cholesterol. Other positions in cholesterol which are sensitive to oxidation include C-20 and C-25.

The (classical) methodology for the determination of COPs in complex matrices involves extraction followed by thin-layer chromatography (TLC) or derivatisation and gas chromatography (GC). Although exposure to alkaline conditions of the samples before analysis leads to breakdown and hence to a lower recovery of some of the COPs [4-6], saponification has been performed by some researchers to also assess the oxidation of cholesteryl esters. Luby et al. [7]
for instance have used TLC for the separation of cholesterol and COPs in saponified butter extracts. Nam et al. [8] have determined a variety of COPs in raw meat using trimethylsilyl derivatisation and GC. Efforts are also made to devise a methodology for rapid and economical analysis of COPs in blood using TLC without as well as in combination with silylation [9].

The presence of COPs in eggs and egg products has been investigated by a number of groups since the 1960s. A review article about the analysis and the biological effects of sterol oxides in foodstuffs has appeared in 1983 [10]. The COPs identified in spray-dried or air-exposed egg yolk the aforementioned major products as well as cholestan-3,5,6-triol, and 5,6-epoxycholestan-3-ol [6, 11]. The latter is reported to derive from cholesterol by reaction with 7-hydroperoxycholesterol or with the hydroxyl radical (OH*) [2]. Some researchers have investigated the prerequisites for the formation of epoxy-cholestanols during spray-drying [12]. Lai et al. [13] have identified the presence of NOx as an important factor in the rapid formation of COPs in gas-fired spray-driers. Tsai and Hudson [5] used high-performance liquid chromatography (HPLC) for the isolation of epoxy-cholestanols from egg powders. In their research MS, NMR and IR spectroscopy were used for identification of the compounds.

Employing extraction, liquid chromatography pre-separation and derivatisation GC, Van de Bovenkamp et al. [4] have determined the presence of 7-hydroxycholesterol, 7-keto-cholesterol and 5,6-epoxy-cholesterol at the sub-μg/g-level in fresh egg yolk. Yang and Chen [14] have used TLC and derivatisation-GC for the determination of COPs in a selection of Chinese egg products. They found that between 1 and 2% of the cholesterol was transformed to 7-β-hydroxycholesterol and 20-hydroxycholesterol by various methods of processing of the eggs.

Kim and Nawar [15] have shown that the rates of cholesterol oxidation in model systems that contain different triacylglycerols vary strongly and that interpretation of the observations is very difficult. It is not the purpose of this chapter to resolve issues of reaction mechanisms and kinetics of cholesterol oxidation in the egg tempera binding medium. Instead, it is our intention to identify marker peaks indicative for cholesterol oxidation products traced by the DTMS-DA methodology. The possibilities for assignment of the marker peaks will be discussed first. Experimental data from DTMSMS of standards and egg tempera paint will be presented subsequently. The spectra will be compared and discussed in the light of mass spectral data on sterols reported in the literature.
5.2 Experimental

5.2.1 Egg tempera samples
For the MSMS and derivatisation experiments on light-aged egg-only tempera a sample of the unexposed egg-only tempera on Melinex (prepared as described in Chapter 1) was exposed to elevated levels of visible light in the MOLART light ageing facility at the Limburg Conservation Institute (SRAL) in Maastricht, The Netherlands. The ageing facility uses 12 Philips TLD-36W/96 fluorescent daylight tubes to illuminate a surface of 1.2 m². The resulting light intensity during the 21 days' exposure of the egg-only tempera sample was 10,200 lx. Perspex (PMMA) filters were used to absorb most of the already low intensities of UV radiation produced by the fluorescent lights. Because the light ageing facility is placed in an air-conditioned room, the temperature and relative humidity could be maintained constant at values of 22°C and 40-44%, respectively.

5.2.2 Reference compounds
Cholesta-4,6-diene-3-one, 5-cholesten-3-one, 5-cholesten-3β-ol (cholesterol), 5-cholesten-3β-ol-7-one, 5β,6β-epoxycholestan-3β-ol, and 5-cholestene-3β,7β-diol were obtained from Sigma-Aldrich Chemie (Steinheim, Germany).

5.2.3 Trimethylsilyl derivatisation
A dichloromethane : ethanol (7:3, v/v) extract of 21-day light-aged egg material were evaporated to dryness in GC auto-injector vials. To the dried extract were added 50 μl per mg sample (weight before extraction) of bis(trimethylsilyl)trifluoroacetamide, containing 1% trimethylchlorosilane (Fluka, Zwijndrecht, The Netherlands) and 50 μl per mg sample (weight before extraction) of pyridine. The reaction vials were flushed with dry nitrogen and placed in an oven at 65°C for 60 minutes. After a subsequent cooling period, the solvent was removed under a gentle stream of dry nitrogen and the residue was redissolved in dichloromethane.
5.2.4 DTMS(MS)

Direct Temperature-resolved Mass Spectrometry (DTMS) was performed as described in the experimental section of Chapter 2. DTMS experiments were performed on a JEOL JMS-SX/SX 102A four-sector instrument of B/E-B/E geometry (which was kindly made available by Prof. Dr. A.R.J. Heck of the Bijvoet-Institute of Utrecht University before it was acquired by the FOM-Institute for Atomic and Molecular Physics). Ions produced in the ion source with a kinetic energy of 8kV were selected by MS1 (first two sectors of the instrument). They were thus passed through the collision cell, which was at ground potential. Helium was used as collision gas. The gas pressure was set to produce a precursor ion signal intensity of 90% of the initial signal intensity on MS2 (no collision gas). As a result, the pressure in the collision cell was $3.5 \times 10^{-3}$ mbar.

5.3 Results and Discussion

5.3.1 DTMS analysis

Discriminant analysis of the spectra of the light ageing series of the egg temperas (results shown in Chapters 2 and 3) indicated which peaks in the mass spectra of the tempera paints increased and which decreased upon light ageing. The peak at m/z 386 was found to decrease upon light ageing. Peaks in the mass window around cholesterol (i.e. m/z 350-450) that increase in intensity upon light ageing are likely to be alteration (oxidation) products of cholesterol. These include m/z 402 (oxocholesterol), 400 (hydroxycholestenone), 384 (cholestenone) and 382 (dehydration product of hydroxycholestenone). The mass thermograms (MTs) of m/z 384, 386, 400 and 402, derived from the DTMS data of 64-day light-aged egg-only tempera are compared in Figure 1. The first peak in the MTs of m/z 384, 400 and 402 (scan number 34) occurs at a higher scan number than that of cholesterol (scan number 30). This is attributed to the higher polarity of the COPs, which causes them to desorb at higher temperatures. At even higher temperatures in the DTMS run other, more abundant and less volatile, material desorbs and pyrolyses, leading to an increase in the signal intensity over a wide mass range. This explains the peaks in the MTs at higher temperatures. It must be noted the low-temperature peaks in the MTs appear in a desorption window
COPs in light-aged egg tempera

Figure 1  TIC and MTs of m/z 386, 384, 400, 402, 416 and 418 derived from the DTMS data of 64-day light-aged egg-only tempera. The MTs are normalised to the maximum intensity of m/z 400; the normalisation factors are given in parentheses.

where the total signal intensity is less than 20% of the maximum (as indicated by the arrow on the total ion current (TIC) trace). Figure 2 shows the summation mass spectrum of 64-day light-aged egg-only tempera over the desorption window of cholesterol (scans 25-40). This spectrum shows high intensities of the peaks m/z 384, 386 and 400. The peaks labelled with “G” derive from glycerolipids (for assignment see Chapter 2, Table 1). The label “BG” indicates a peak that originates from background signal (chemical noise).
Table 1 summarises the possibly cholesterol-related peaks in Figure 2 and gives their assignment. This table only specifies assignments that seem likely on the basis of the literature on cholesterol oxidation in egg yolk, or in exceptional cases on the basis of pathways of progressed oxidation of egg-yolk COPs. Hydroperoxides are not included in the table, given their presumed short life-time in their chemical matrix and the instability of their molecular ions under EI conditions. A specified (in parentheses) example of the structure of the ion [16] or molecule (A- and B-rings only) mentioned in the second column of Table 1 is given in the third column of the table. As an aid for the interpretation of the table Scheme 1 shows the numbering of the cholesterol skeleton.

![Scheme 1](image1)

**Figure 2** Partial (m/z 250-420) summation mass spectrum of the cholesterol desorption window (scans 25-40) for 64-day light-aged egg-only tempera. Peaks labelled with G derive from glycerolipids; BG from background (chemical noise).

The first three peaks listed most probably derive from cholesterol and are assigned as EI fragmentation products. The same applies for the peak at m/z 368, although there are two other possible sources, viz. cholestadiene and a fragment due to loss of a fatty acid neutral from a cholesterol ester molecular ion. The latter is discussed in Chapter 3. Cholestadiene is not classified as a COP, but rather as a dehydration or pyrolysis product of cholesterol [2]. DTMS analysis of free cholesterol indicated that the pyrolytic formation of cholestadiene from cholesterol is minimal during the DTMS experiment. Catalytic dehydration in the presence of large amounts of inorganic paint substances, however, cannot be excluded.
Table 1 Assignment of intense peaks in the summation mass spectrum of the cholesterol desorption window of light-aged tempera.

<table>
<thead>
<tr>
<th>m/z</th>
<th>Compound/fragment</th>
<th>Structure of compound / fragment</th>
</tr>
</thead>
<tbody>
<tr>
<td>275</td>
<td>$M^+\cdot C_7H_{11}O$ (C1-7) from cholesterol</td>
<td><img src="image1" alt="Structure of compound" /></td>
</tr>
<tr>
<td>301</td>
<td>$M^+\cdot C_8H_{13}$ (C22-27) from cholesterol</td>
<td><img src="image2" alt="Structure of compound" /></td>
</tr>
<tr>
<td>353</td>
<td>$M^+\cdot (H_2O + CH_3^+)$ from cholesterol</td>
<td><img src="image3" alt="Structure of compound" /></td>
</tr>
<tr>
<td>368</td>
<td>$M^+\cdot H_2O$ from cholesterol $M^+\cdot FA$ from cholesterylester</td>
<td><img src="image4" alt="Structure of compound" /></td>
</tr>
<tr>
<td>371</td>
<td>$M^+\cdot CH_3^+$ from cholesterol</td>
<td><img src="image5" alt="Structure of compound" /></td>
</tr>
<tr>
<td>382</td>
<td>cholestadienone $M^+\cdot H_2O$ from cholest-5-en-3-ol-7-one</td>
<td><img src="image6" alt="Structure of compound" /></td>
</tr>
<tr>
<td>384</td>
<td>cholestenone $M^+\cdot H_2O$ from cholest-5-en-diols $M^+\cdot H_2O$ from 5,6-epoxy-cholestan-3-ol</td>
<td><img src="image7" alt="Structure of compound" /></td>
</tr>
<tr>
<td>386</td>
<td>Cholesterol (cholest-5-en-3-ol)</td>
<td><img src="image8" alt="Structure of compound" /></td>
</tr>
<tr>
<td>400</td>
<td>3-hydroxycholest-5-en-7-one (7-hydroxycholest-5-en-3-one) $M^+\cdot H_2O$ from m/z 418</td>
<td><img src="image9" alt="Structure of compound" /></td>
</tr>
<tr>
<td>402</td>
<td>5,6-epoxy-cholestan-3-ol cholest-5-en-3,7-diol cholest-5-ene-3,20-diol cholest-5-ene-3,25-diol (M$^+\cdot H_2O$ from cholestane-3,5,6-triol)</td>
<td><img src="image10" alt="Structure of compound" /></td>
</tr>
<tr>
<td>416</td>
<td>3,25-dihydroxycholest-5-en-7-one</td>
<td><img src="image11" alt="Structure of compound" /></td>
</tr>
<tr>
<td>418</td>
<td>5,6-epoxycholestan-3,25-diol 5-cholesten-3,7,25-triol</td>
<td><img src="image12" alt="Structure of compound" /></td>
</tr>
</tbody>
</table>
On the basis of molecular mass cholestanedienone is a suggestion for the peak at m/z 382. Smith [2] has suggested three pathways for the oxidative formation of cholesta-4,6-dien-3-one from cholesterol. One of these involves successive oxidation, isomerisation and dehydration of cholest-5-en-3,7-diol. Another is based on reaction of cholest-5-en-3-one with singlet oxygen (\(^{1}\)O\(_2\)), and suggests 5-hydroperoxycholest-6-en-3-one as an intermediate. Experiments by Chicoye [6] have indicated that cholest-5-en-3-ol-7-one is transformed to cholesta-3,5-dien-7-one under conditions of saponification (81°C in KOH solution). Thus, in the case of the lead white tempera, the basicity of the lead hydroxy carbonate pigment (Pb\(_2\)(OH)\(_2\)CO\(_3\)) may account for the high intensity of the m/z 382 peak in the DTMS spectra of light-aged lead white tempera (see Chapter 3). In relation to this it must be remarked that m/z 400 is the highest peak in the cholesterol mass window of the DTMS spectra of lipid extracts of light-aged lead white tempera (data not shown). This indicates that transformation of ketocholesterol to cholestanedienone takes place in the desorption process on the DTMS probe.

The peak at m/z 384 may derive from the molecular ion of 5-cholesten-3-one, which can be formed from cholesterol upon reaction with \(^{3}\)O\(_2\) [2]. As mentioned before, 3-hydroxycholest-5-en-7-one is another well-known (abundant) COP. Attribution of the peak at m/z 400 to this compound is therefore plausible. The possibility of a minor contribution from 7-hydroxycholest-5-en-3-one is not excluded as this is one of the structures involved in the aforementioned pathway for the formation of cholesta-4,6-dien-3-one from cholest-5-en-3,7-diol.

Two of the COPs mentioned in the introduction have a molecular weight of 402 Da, viz. 5,6-epoxy-cholestan-3-ol and cholest-5-en-3,7-diol. Clearly, other cholestendiois may also be responsible for the peak at m/z 402. Cholest-5-ene-3,20-diol and cholest-5-ene-3,25-diol have been detected in egg products [17].

If the possibility of multiple oxygenation of cholesterol is taken into account, COPs with molecular weights between 414 and 420Da can be expected. Therefore, extra attention was paid to these masses in Figure 2. As a result two of the peaks in this mass window could be tentatively assigned to COPs on the basis of their MTs, viz. m/z 416 and 418. The MTs of these peaks are also shown.
in Figure 1. The shift of the local maximum of these MTs (marked with arrows) with respect to the maximum of the MTs of m/z 400 and 384 is again attributed to the higher polarity of these compounds as a result of their higher degree of oxygenation. The MTs of the other peaks between m/z 414 and 420 (data not shown) do not show a local maximum in the desorption window of cholesterol. Based on the extensive literature on the oxidation of cholesterol, progressed oxidation can occur. The two most-preferred sites on the sterol skeleton for progressed oxidation are the 7- and the 25-position. Thus, 5,6-epoxycholestan-3,25-diol and 5-cholesten-3,7,25-triol are suggested as the most probable COPs for m/z 418 and 3,25-dihydroxycholest-5-en-7-one for m/z 416. The formation of the latter two products by autoxidation of 5-cholesten-3,25-diol has been suggested by Smith [18]. Similarly, autoxidation of the well-known COP 5-cholesten-3-ol-7-one at the 25-position was suggested to explain the formation of 3,25-dihydroxycholest-5-en-7-one.

As indicated in Table 1, loss of water from molecular radical cations is also part of the attribution of the peaks in Figure 2. Thus, water loss from the M⁺ of ketocholesterols can contribute to the intensity of the peak at m/z 382. It must be noted however that the relative intensity of m/z 382 in the 16eV DTMS spectrum of cholest-5-en-3-ol-7-one is less than 20% of the base peak (M⁺). In the 16eV DTMS spectrum of cholest-5-en-3,7-diol, the M⁺-H₂O peak (m/z 384) is the base-peak and the molecular ion (m/z 402) shows a relative intensity of 15% (data not shown). It must be assumed that the spectra of other cholestenediols will also show a significant peak at m/z 384. 5,6-Epoxycholestan-3-ol produces M⁺-H₂O ions at a relative intensity of 20% of the molecular ion (base-peak) and hence will also contribute to the signal intensity at m/z 384.

The ion at m/z 402, in turn, can partly originate from water loss of a COP with molecular weight 420. Cholestane-3,5,6-triol, which is known as a hydration product of 5,6-epoxy-cholestan-3-ol [2] and has been detected in an 8-year old egg yolk sample [17]. Water loss from the molecular ion of this compound is highly probable due to the presence of three hydroxyl groups and may even explain the absence of a peak at m/z 420 in Figure 2. Hence, the presence of small quantities of the compound in the light-aged tempera sample cannot be excluded. Because Figure 2 indicates the presence of COPs with a molecular weight of 418Da, which probably contain more than one hydroxyl group, fragmentation of the molecular ions of these compounds by loss of water may also partly account for the m/z 400 peak.
5.3.2 DTMSMS of COPs

Although it is evident that m/z 386 originates from cholesterol, it was subjected to DTMSMS analysis as a (matter of) test for the DTMSMS methodology. Figure 3A shows the DTMSMS spectrum of peak m/z 386 from sample of 21-day light-aged egg-only tempera. Comparison with the 70 eV electron impact ionisation spectrum of cholesterol (Figure 3B) indicates that peak m/z 386 in the DTMS spectra of egg-only tempera can indeed be attributed to cholesterol. Characteristic peaks for the fragmentation of cholesterol are observed in both spectra. These include peaks at m/z 371 (M" – CH₃), m/z 368 (M" – H₂O), m/z 353 (M" – CH₃ – H₂O), m/z 301 (loss of C22-27), m/z 275 (loss of C1-7) [16, 19], m/z 273 (loss of C20-27 +H), m/z 231 (loss of C16-17+C20-27+H) and m/z 213 (loss of C16-17+C20-27+H+H₂O).

Figure 3 DTMSMS spectrum of peak m/z 386 from 21-day light-aged egg-only tempera (A) and DTMS spectrum (EI, 70 eV) of cholesterol (B).
Figure 4A shows the DTMSMS spectrum of the m/z 400 peak from light-aged (21 days at 10,000 lx) egg-only tempera. Comparison of this spectrum with the DTMSMS spectrum of a 5-cholesten-3-ol-7-one standard (Figure 4B) shows great similarities. M/z 385 and m/z 382 are attributed to loss of a methyl radical and a water molecule, respectively. The peak at m/z 367 is the combined result of these two fragmentations. Fragmentation at the side chain (bond C17-20) leads to the formation of m/z 287 by loss of a CgH17' radical. The peak at m/z 205 derives from a rearrangement fragmentation in the C-ring, that breaks the bonds C8-14 and C12-13 and involves the transfer of one hydrogen. The same fragmentation combined with the loss of water results in m/z 187. Each of these peaks in the MSMS spectrum of the standard compound is also present in Figure 4A. A few peaks in the DTMSMS spectrum of m/z 400 from the light-aged egg-only sample do not appear in the spectrum of the standard compound (e.g. 297 and 315). This suggests that other components must be present in the light-aged tempera, which form ions of m/z 400. Possibly fragment ions with m/z 400 that result from water

Figure 4  DTMSMS spectra of peak m/z 400 from 21-day light-aged egg-only tempera (A) and 3-hydroxycholest-5-en-7-one (B).
loss of COPs with molecular weight 418 Da contribute to the peak. Differences in internal energy of the ions may account for differences in relative intensities of important peaks in the mass spectra. The presence of a peak at m/z 192 in the 70eV EI spectrum of 5-cholesten-3-ol-7-one confirms this notion.

**Figure 5** DTMSMS spectra of peak m/z 402 from 21-day light-aged egg-only tempera (A), 5,6-epoxy-cholestan-3-ol (B) and cholest-5-en-3,7-diol (C).
The DTMSMS spectrum of peak m/z 402 from the light-aged egg-only tempera sample (21 days) shown in Figure 5A closely resembles that of the molecular ion of 5,6-epoxy-cholestan-3-ol shown in Figure 5B. Differences between the spectra may derive from a variety of factors. First of all there may be a minor difference in internal energy of the precursor ions. Then, Tsai et al. [5] have shown that configurational differences in the structure of 5,6-epoxy-cholestan-3-ol are expressed in the mass spectra. Although discrimination between the two epimers is not possible on the basis of the spectra presented, 5,6-epoxy-cholestan-3-ol is positively identified in the light-aged egg-only tempera. It must be remarked that the 5β,6β-epoxide is thermodynamically more stable than the 5α,6α-epoxide and this is used to explain the higher relative abundance of the β-epoxide [13]. 5,6-Epoxycholestersols are formed upon reaction of cholesterol with active oxygen species such as singlet oxygen (\(1^1\text{O}_2\)) and hydroperoxide [18]. The high abundance of 5,6-epoxycholesterolms may thus be explained by interaction between cholesterol and hydroperoxide functionalities of (oxidised) egg glycerolipids. As mentioned earlier 5-cholesten-3,7-diol is another likely candidate to (partly) account for the peak m/z 402. The DTMSMS spectrum of the molecular ion of this compound is shown in Figure 5C. The lack of specificity of the DTMSMS spectrum and the strong contribution of 5,6-epoxy-cholestan-3-ol to the spectrum in Figure 5A do not allow a partial attribution of the peak at m/z 402 to 5-cholesten-3,7-diol. Recall, however that cholest-5-en-3,7-diol shows a relatively small molecular ion peak m/z 402 under 16eV EI conditions. Therefore, the cholest-5-en-3,7-diol will mainly contribute to the intensity at m/z 384.

The MSMS peaks from m/z 384 in the spectra of 5-cholesten-3-one (M\(^{+}\)), 5-cholesten-3,7-diol and 5,6-epoxy-cholestan-3-ol (both M\(^{+}\) – H\(_2\)O) did not allow the unambiguous identification of the structure of the precursor ion.

The question of the identity of the presence of cholestenediols was also investigated by DTMS analysis of trimethylsilyl (TMS) derivatised standards and samples. DTMS analysis of silylated COPs, such as 5-cholesten-3-ol-7-one, 5-cholesten-3-one and 5,6-epoxy-cholestan-3-ol, indicated that epoxy- and keto-functionalities are not silylated. The spectrum of TMS derivatised cholest-5-en-3,7-diol showed a molecular ion at m/z 546 and a base peak at m/z 456, indicating that the loss of trimethylsilanol (HOSi(CH\(_3\)_3)) is an important feature in the spectra of silylated cholestenediols. The observation of a peak at m/z 546 and an intense peak at m/z 456 in the partial (cholesterol desorption window) DTMS spectrum of the silylated extract of 21-day light-aged egg-only tempera (data not shown) indicate the presence of COPs with two hydroxyl functionalities on a cholestene skeleton in the light-aged sample. Furthermore, in a similar way, the
observation of peaks at m/z 472 and 474 in the same spectrum confirmed the presence of hydroxycholestenone (probably cholest-5-en-3-ol-7-one) and 5,6-epoxy-cholestan-3-ol in the 21-day light-aged egg-only tempera sample.

### 5.4 Concluding remarks

The results presented show that DTMSMS and the combined use of DTMS with trimethylsilyl derivatisation are useful tools for the characterisation of molecular changes in light-aged egg tempera samples. Using DTMSMS, 5,6-epoxycholestan-3-ol and 3-hydroxycholest-5-en-7-one were positively identified in light-aged egg tempera binding medium. The former compound is likely to have been formed as a result of interaction between peroxidised glycerolipids and cholesterol. DTMS of trimethylsilyl derivatised light-aged egg-only tempera allowed the identification of cholestenediol. Hence the assignment of cholesterol-related m/z peaks in Chapter 2 is confirmed and refined. The markers identified are useful in tempera paint-based dosimetry.

As the oxidation of cholesterol develops early in the life-time of egg tempera paint and is accelerated by the presence of inorganic pigments, cholesterol itself is not a good marker for egg as binding medium in paintings. Alternatively, cholesterol oxidation products may be better markers, provided that they survive in the course of time.

### References


