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Abstract

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Keywords	gas chromatography – mass spectrometry; fatty acids; textile; pig; decomposition
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Seasonal variation of fatty acid profiles from textiles associated with decomposing pig remains in a temperate Australian environment

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Abstract

A methodology to examine the human post-mortem decomposition process has been developed through the monitoring of chemical changes to decomposition fluids absorbed by clothing. Model surface deposition using clothed pigs were established during summer and winter seasons in a temperate region of Australia. Three clothing materials were investigated: cotton, polyester and cotton-polyester. Lipid decomposition products were extracted from the textiles and the fatty acid composition measured as a function of surface deposition time using gas chromatography – mass spectrometry (GC-MS). Two derivatisation methods for the fatty acids were compared to establish the most effective approach and it was established that a trimethylsilylation derivatisation method is the optimal preparation technique. The summer trials revealed two rates of transformation of fatty acids from unsaturated to saturated forms, with a faster rate of change occurring earlier in the trials. A different pattern of behaviour was observed for the fatty acids detected during the winter trial, with a decrease in saturated fatty acids initially observed, followed by the conversion of unsaturated to saturated fatty acids until the end of trial. The initial change observed during the winter trial was attributed to a dehydrogenation process caused by microbiological enzymatic activity. The study has demonstrated the feasibility of examining lipid decomposition products

collected in clothing from surface depositions to provide insight into the conditions and length of deposition.

Keywords: gas chromatography – mass spectrometry; fatty acids; textile; pig; decomposition.

1. Introduction

Clothing can provide a valuable source of evidence in a variety of crime scenes. When associated with human remains, the collection and examination of clothing may assist investigators in determining the nature of the death [1]. Human remains undergo a decomposition process that results from the breakdown of tissue. The natural decomposition process involves changes to lipids, proteins, and carbohydrates, and the by-products form the major components of decomposition fluid. The resulting compounds can become embedded in associated clothing, often leading to observable discolouration of the material and can influence the degradation of the clothing material [2]. The fact that decomposition fluid can be retained in clothing, rather than leaching into an environment such as soil, provides an opportunity to gather crucial information about the decomposition process through the analysis of the retained compounds.

The breakdown of lipids during the decomposition process is known to produce a mixture of free fatty acids [3,4]. The dominant lipid type in adipose tissue is the triacylglycerols. Following death, triacylglycerols undergo hydrolysis due to the presence of intrinsic tissue lipases, producing a mixture of unsaturated and saturated free fatty acids. The nature of the reactions of the fatty acids depends on the type of environment in which the decomposition occurs. As a decomposing body is exposed to reducing conditions, hydrolysis of the resulting fatty acids is more likely than an oxidation process. In the anaerobic environment produced as a result of microbiological activity, the

mixture of unsaturated and saturated fatty acids produced during the post-mortem hydrolysis process undergo further hydrolysis and hydrogenation, thus changing the resulting fatty acid composition. For instance, the hydrogenation of oleic and palmitoleic acids yield stearic and palmitic acids, respectively.

Gas chromatography – mass spectrometry (GC-MS) is an established method for the analysis of fatty acids and its use has been demonstrated for the quantitative identification of post-mortem decomposition products, primarily using pig as a model for human decomposition processes [5,6]. One study has demonstrated that lipids can be extracted from textiles recovered from a model pig burial where the animals were clothed in natural and synthetic clothing materials [7]. GC-MS was employed in the study to characterise the fatty acid composition of decomposition products extracted from textiles with the aim to examine the effect on textile preservation. The study showed that the lipid composition could be monitored with deposition time, demonstrating a quantitative approach to an understanding of the decomposition process of clothed remains.

In the current investigation, GC-MS has been employed to examine the fatty acids produced post-mortem and absorbed by clothing in a field study using pig remains to model the decomposition process. The decomposition process is known to be influenced by the environment and climatic conditions were compared for this study through the establishment of decomposition trials beginning during summer and winter seasons. Fatty acids require a derivatisation process prior to GC-MS analysis and two derivatisation methods have been compared to establish the most effective approach for the samples collected for this study. Clothing specimens were collected over the periods of the trials and the fatty acid composition was monitored to compare the lipid decomposition processes when remains are deposited during different seasons in a temperate Australian environment.

2. Materials and methods

2.1 Textile preparation

A field site located in western Sydney, Australia was used for establishing the trials. The area is characterised as woodland with a large presence of eucalyptus trees and the topsoil at the location is acidic (pH 4 – 5). Pig (*Sus Scrofa*) remains were clothed in three different materials: 100% cotton t-shirts (Alpha brand, Kmart, Australia), 100% polyester briefs (Alpha brand, Kmart, Australia) and cotton-polyester blend socks (Tiny Little Wonders, Kmart, Australia). The remains of three separate pig carcasses were established for each trial. The remains were protected using a steel grid metal cage in order to allow insects to access to the remains, while preventing scavenging from larger animals (illustrated in Figure 1). Three control sites were created, consisting of identical clothing to those that used on the pig carcasses, but placed directly on the soil surface adjacent to the pig set-ups.

Three trials were established with different start dates: the first 'summer' trial commencing January 2013 for 365 days; the second 'summer' trial commencing January 2014 for 369 days; a 'winter' trial commencing July 2014 for 565 days. The ambient temperature (°C) and rainfall (mm) for the trial site were monitored using a HOBO® No Remote Communication weather station base with sensors (OneTemp) set-up in the field adjacent to the decomposing carcasses. Table 1 lists the relevant weather data for the trials. The visual changes observed to the pig remains during decomposition are reported in Table 2 and were categorised using established stages of decomposition (i.e. fresh, bloat, active decay, advanced decay, dry remains) [7-9].

Three 5 cm × 5 cm swatches of the three different textile types were collected from the control sites and each of the pigs at determined sampling days using sterilised scissors. Sampling was carried out on 19 days for the first summer trial, 15 for the second summer trial and at 19 time points for the winter trial. Sampling was conducted weekly during the initial stages of decomposition, and then less frequently as the remains entered the later stages of decomposition. The samples were packaged in paper envelopes, placed in individually labelled paper bags and stored in a cooler for transport. Upon arrival in the laboratory the samples were air dried in a fume hood to prevent any further bacterial and fungal growth. Any adhering soil or vegetation was removed from the samples once dry. The textiles were stored in paper bags at -18 °C until analysis.

2.2. Fatty acid methyl ester derivatisation method

The first method used to prepare the samples for GC-MS analysis was a modified version of the direct fatty acid methyl ester (FAME) synthesis reported by O'Fallon *et al.* [10]. The textile sections (3 cm × 3 cm) were placed in scintillation vials with 8 mL high performance liquid chromatography (HPLC) grade chloroform (Burdick & Jackson, USA). The samples were sonicated for 30 min, vortexed for 2 min and then left for 12 h at 4°C in order to extract fatty acids. Following extraction, the textile squares were removed from the scintillation vials. A 1.5 mL aliquot of the extracted fatty acid solution was added to a Pyrex™ screw cap tube and 6.3 mL methanol (Burdick & Jackson, USA) was added to the tube followed by 700 µL 10 N KOH (Sigma-Aldrich, USA). Each sample was shaken well before incubation at 55°C for 1.5 h, with additional vigorous shaking for approximately 5 s every 20 min in order to dissolve and hydrolyse the sample. Each sample was cooled in a cold-water bath before adding 580 µL sulfuric acid (Sigma-Aldrich, Germany) and placed back onto the heating block for an additional 1.5 h at 55°C, again with vigorous shaking every 20 min. Each sample was then

cooled in a cold-water bath and 3 mL hexane (SK Chemicals, Korea) added. Each Pyrex™ tube was vortexed twice before centrifugation at 3000 rpm for 5 min. 200 µL aliquots of the top layer from each sample tube was removed by pipette and added to a GC vial. 100 µL of an internal standard (1000 ppm nonadecanoic fatty acid methyl ester, Sigma Aldrich, USA) was also added to each vial and the volume made up to 2 mL using HPLC grade hexane and the GC vial inverted. The remainder of the top layer of the sample was added to a scintillation vial for storage in a freezer at -18°C.

2.3 Trimethylsilylation derivatisation method

A second derivatisation method was adapted from Folch *et al.* [11] and involved the silylation of the fatty acids. For this approach, 3 cm x 3 cm sections of the textile samples were placed into individually labelled scintillation vials and 8 mL chloroform was added. The samples were sonicated for 30 min, vortexed for 2 min and then left for 12 h at 4°C as with the previous method. The textile squares were removed from the scintillation vials. 1.8 mL of the extracted fatty acid solutions were transferred to screw-top culture tubes, to which 0.2 mL N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) with 1 % trimethylchlorosilane (TMCS) and 0.2 mL of internal standard (1000 ppm nonadecanoic acid, Fluka Analytical, Switzerland) was added. The samples were vortexed for 1 min and heated for 15 min at 70°C using a heating block. Upon cooling, the samples were transferred GC vials.

2.4 Gas chromatography – mass spectrometry

GC-MS analysis was performed using an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass selective detector (Agilent Technologies, Australia). An HP-5MS (5 % diphenyl, 95 % dimethyl polysiloxane) ultra-inert column (30 m × 0.25 mm ID, 0.25 µm film thickness, J & W Scientific, USA) was used during the analysis. Helium (high purity, BOC, Australia) was used as the

carrier gas at a constant flow rate of 1.2 mL/min. All injections were performed in split mode with a split ratio of 25:1 and an injection volume of 1 µL using an Agilent 7683B Series autosampler.

For the FAME derivatised samples, the oven temperature program was as follows: Increased from 135 – 180°C at 4°C/min and held for 4 min; increased to 200°C at 4°C/min and held for 5 min; increased to 250°C at 4°C/min and held for 10 min. The total analysis time was 52.75 min. The mass selective detector was operated in full electron ionisation (EI) scan mode was with an emission current of 30 µA, a scan time of 0.50 s/scan and a mass range of 50 – 450 m/z. A fatty acid methyl ester mix (C5 – C23) purchased from Supelco (USA) was used to confirm the identification of peaks.

For the samples prepared using the trimethylsilylation (TMS) derivatisation method, the initial oven temperature was 50°C, this was increased to 190°C at 15°C/min and held for 4 min; increased to 200°C at a 1°C/min and held for 3 min; increased to 240°C at a rate of 15°C/min and held for 5 min. The total run time was 34 min. A full scan was performed with an emission current of 30 uA and a scan time of 0.50 s/scan. The scan used a range of 50 – 450 m/z ratios. Individual fatty acid standards of palmitic, myristic, oleic, palmitoleic, linoleic and stearic acids (all from Sigma Aldrich, USA) were derivatised using the TMS method for confirmation of the identification of peaks. For the control textile samples, no fatty acids were detected for any sampling day for each of the trials. GC-MS data analysis was performed using Agilent MSD ChemStation (version E 02.01.1177, Agilent Technologies, Australia) and Microsoft Excel.

3. Results and discussion

3.1 Comparison of derivatisation methods

The concentrations of fatty acids detectable by applying the FAME and TMS derivatisation methods were compared using material extracted from cotton specimens. The analyses were carried out on

cotton collected on day 21 of the first summer trial. The fatty acids measured and the relative amounts detected for this sampling day are illustrated in Figure 2. The TMS method is shown to yield notably higher overall concentrations of fatty acids compared to the FAME method. Previous investigations of the poorer recoveries associated with FAMEs used for the analysis of fatty acids have indicated that this may be associated with competing hydrolysis reactions [12]. For the current study, the hydrolysis due to the presence of residual water in the samples is a likely source of the low recoveries observed. The TMS derivatised samples also provide higher individual fatty acid concentrations compared to those values obtained for the FAME derivatised samples. The component percentages of the fatty acids per sampling day were also investigated and the results are illustrated in Figure 3. The ratios of the respective fatty acids are comparable for both derivatisation methods for the range of sample times.

The FAME method was found to be more labour intensive and involved two 1.5 h heating periods, where the samples were required to be shaken every 20 min, making the total derivatisation method extensive (approximately 5 h per sample). By contrast, the TMS method was less time consuming, with a total derivatisation time of approximately 20 min per sample. Due to the higher sensitivity and reduced preparation time, the TMS method was chosen as the preferred derivatisation method for the study.

3.2 Summer trials

The individual fatty acid concentrations measured for each of the textile specimens collected on all sampling days of the summer trials are presented in Figure 4. A comparison of the total amount of fatty acids detected for each sampling day reveals that the first appreciable appearance of fatty acids is observed by days 4 and 6 for each textile type for the first and second summer trials,

respectively. These sampling days correlate with the end of the bloat stage and the early part of the active decay stage recorded for each of the trials. For cotton collected during the first summer trial appreciable quantities of fatty acids were detected in specimens from sampling days during the active and advanced decomposition stages, with reduced amounts noted for the later sampling days occurring during the dry stage. Similar observations were made for the fatty acids detected in polyester and cotton-polyester specimens. One exception was seen with the amount of palmitic and stearic acids in the cotton-polyester specimens from summer trial 1 on days 59, 149 and 212, which showed an increased abundance. This increase could be attributed to the absorbent nature of the material.

The patterns of fatty acids detected for each of the textile types collected during the second summer trial differed from those observed for the first trial. Notable amounts of fatty acids were measured for sampling days during the active and early advanced stage, but substantially declined after day 45 for each textile type. Although the average temperatures throughout both the summer trials were similar (Table 1), the total rainfall recorded during the first summer was notably higher than that recorded the following year. The wetter environment appears to be a factor in the more extended detection of fatty acids in the first summer trial. The elongated period of fatty acid detection could be due to the longer decomposition process undergone by the pig remains. The wetter environment caused the remains to remain in the advanced decay stage for a longer period than the pigs in the drier summer did (Table 2). It was noted that the pigs placed in the second summer progressed into a skeletal stage, meaning they were losing most of the soft tissue faster than their summer 1 counterparts. These results are consisted with literature stating that a higher level of humidity leads to slower rates of cadaver degradation [13]. The wetter environment were also most likely aiding in preserving the tissue embedded into the textile and therefore allowing

fatty acids to be detected for longer than the textiles that dried in the absence of heavy rainfall in summer trial 2 by keeping the fatty acids hydrated.

One means of examining the decomposition process using fatty acid concentrations is to monitor the transformation of unsaturated to saturated fatty acid concentrations that results from hydrogenation reactions. Such an approach has been used to monitor the formation of the decomposition product adipocere, which is comprised of a mixture of fatty acids [6,14]. Earlier investigations divided the process of adipocere formation into three stages based on the amount of saturated fatty acids present and the % of saturated fatty acids at each stage has been estimated as early (40–60%), intermediate (70–90%) and advanced (>90%). A similar process has been applied for this study to estimate the extent of change in fatty acid composition at different times during the trials.

Figure 5 illustrates the percentage of saturated fatty acids determined for the samples from textiles collected on each sampling day of the three trials. An examination of the results obtained for each summer trial reveals an increase in the presence of saturated fatty acids and, therefore, a corresponding decrease in unsaturated fatty acid over the lifetime of the trials (one-way ANOVA ($p = 0.05$) applied). The plot for the first summer trial for cotton indicates a sharp increase in the percentage of saturated fatty acids formed early in the trial (49% on day 4) until day 31 (78%), after which the change slows and remains so until the end of the trial (99% at day 212) (Figure 5a). This indicates that the fastest rate of transformation of fatty acids occurs in the earlier stages of the trial. The fatty acid composition results also allow the decomposition stages to be estimated as early for days 4-13, intermediate for days 31-59 and advanced for days 149-212. A similar trend is observed for the cotton collected during the second summer trial (Figure 5b). That is, a sharp increase in the formation of saturated fatty acids from the beginning of the trial followed by a reduced

transformation is also observed. The values of % saturated fatty acids also correspond to early, intermediate and advanced stages of decomposition.

Similar trends are observed for the samples collected from polyester and cotton-polyester specimens for both summer trials (Figure 5). Each shows a faster increase in % saturated acids at the beginning of the trial followed by a slowing of the change in fatty acid transformation. An exception is for samples collected from polyester (Figure 5e) during the second summer trial, where no fatty acids were detected on a number of sampling days. Increased rainfall was recorded for the period immediately prior to sampling days that showed no fatty acids were contained in the polyester. While the more absorbent cotton appears to retain fatty acids even with increased rainfall, the fatty acids are apparently washed from the surface of the polyester prior to sampling on those days.

3.3 Winter trial

Figure 6 illustrates the individual fatty acid concentrations measured for each of the textile specimens collected on the sampling days of the winter trial. It is not until day 32 that fatty acids are detected in notable quantities for specimens collected during the winter trial, indicating a slowing of the decomposition process when compared to the summer trials where fatty acids are produced within days. Most of the fatty acid is detected on sampling days that correlate with the active decay stage (days 37-79), which occurred during the later days of the winter trial.

The percentage of saturated fatty acids calculated for the samples from textiles collected on each sampling day of the winter trial is also shown in Figure 5 c and f. The conversion of unsaturated to saturated fatty acids produces a different trend during the winter trial compared to both the summer trials. Rather than a continual increase in the formation of saturated fatty acids throughout

the trial, the winter trial produces a decrease in saturated fatty acids from the beginning of the trial until day 65, after which the % saturated fatty increases until the end of the trial.

An explanation for the initial decrease followed by an increase in the relative amount of saturated fatty acids throughout the winter trial is obtained via an inspection of relative concentration changes observed for specific fatty acids. The major unsaturated fatty acid found in adipose tissue is oleic acid. For both the summer trials, the concentration of oleic acid decreases throughout the respective trials. Figure 7 illustrates the relative concentration of oleic acid as a function of sampling time for each trial. Both summer plots demonstrate a faster decrease in concentration during the earlier part of the trial until advanced decay is observed, followed by a slower change observed corresponding to sampling during an advanced state of decomposition

A notably different behaviour is observed for the winter trial, with increasing amounts of oleic acid observed until sampling day 65 occurring during active decay, after which the concentration continues to decline until the end of the trial (Figure 7). The delay in the overall decomposition process due to the colder climatic conditions has revealed evidence of additional processes associated with the fatty acids from the decomposing adipose tissue. A likely mechanism for the initial increase in the unsaturated oleic acid is the dehydrogenation of stearic to oleic acid due to an oxidation process produced by microbial enzymes present in the early post-mortem stage. This mechanism was established in earlier experimental studies where stearic acid was converted to oleic acid using *Corynebacterium diphteriae* [15,16]. Further support for this mechanism in the current study is provided by monitoring the change to the stearic acid concentrations over the lifetime of the seasonal trials. The plots illustrated in Figure 7 show the corresponding stearic acid changes and support the transformation of stearic acid to oleic acid followed by the hydrogenation process later in the winter trial.

The faster nature of the decomposition process observed for the summer trials does not allow this mechanism to be detected. An earlier study of the formation of adipocere in adipose tissue from pig and human remains immersed in a water environment demonstrated similar trends in the relative concentrations of oleic and stearic acids during the earlier stages of decomposition [6]. In most studies involving the post-mortem monitoring of unsaturated and saturated fatty acid concentrations reported, the focus has not been on the very early stages and, hence, this dehydrogenation process is not generally detected. Further investigation into microbiological activity early in the decomposition process is warranted to elucidate the source of the proposed enzymatic process.

4. Conclusions

GC-MS has been employed to examine the changes in fatty acid composition of decomposition products extracted from three textile types in contact with pig remains during surface deposition established during summer and winter seasons in a temperate region of Australia. Two derivatisation methods were compared to establish the most effective method for quantifying fatty acids in adipose tissue and its decomposition products. A trimethylsilylation derivatisation method was shown to have a higher sensitivity and be a more efficient method for fatty acid analysis of the decomposition products of interest in this study.

An examination of the fatty acid composition of the decomposition products extracted from three textile types (cotton, polyester and a cotton-polyester blend) collected during the summer trials revealed different transformation rates from unsaturated to saturated fatty acids. By monitoring the % saturated fatty acid composition, two periods were identified with clearly different rates of fatty acid transformation: a period of rapid change followed by a period of slower change until the end of the trial. By carrying out a trial beginning during the winter season, a delayed decomposition

process was evident, with fatty acids appearing in detectable quantities only on a later sampling day for the winter trial compared to that observed for the summer trials. Of significance for the winter trial is the observation of a decrease in saturated fatty acids in the early detection stages, subsequently followed by the expected transformation of unsaturated to saturated fatty acids due to a hydrogenation process. By focussing on the changes in oleic and stearic acid concentrations, a dehydrogenation mechanism is proposed that accounts for the initial process during the winter trial. The textile type was not found to significantly influence the rates or nature of the transformation processes associated with fatty acids.

The results of this study have demonstrated that the decomposition behaviour of clothed remains in seasonal surface depositions may be understood by examining the chemical changes to adipose tissue lipids. The monitoring of the fatty acid composition of extracted decomposition products using GC-MS enables a quantitative approach to be taken to characterising the decomposition process. The establishment of how climatic conditions specifically influence the decomposition of clothed remains will be of assistance to forensic practitioners investigating time since the deposit of decomposed remains.

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Table 1. Temperature and rainfall data for trials.

dates	average daily temperature (°C)	maximum temperature (°C)	minimum temperature (°C)	total rainfall (mm)
Jan–Feb 2013	23	47	14	274
Mar–May 2013	17	30	15	98
Jun–Aug 2013	11	24	2	64
Sep–Nov 2013	18	37	6	171
Dec–Feb 2014	23	29	17	104
Mar–May 2014	18	23	11	85
Jun–Aug 2014	11	16	7	108
Sep–Nov 2014	19	29	13	83

Table 2. Decomposition stages observed for sampling days.

trial	fresh	bloat	active	advanced	dry
1st summer	0-1 days	2-3	4-20	21-183	184-356
2nd summer	0-2	3-5	6-9	10-99	100-369
winter	0-12	13-36	37-78	79-209	210-565

Figure legends

Figure 1. Pig trial cage set-up.

Figure 2. Concentrations of selected fatty acids obtained for FAME and TMS derivatised samples collected from cotton at day 21 post-mortem of the first summer trial. Error bars represent standard deviation based on 9 replicate samples.

Figure 3. Mean % fatty acid per sampling day for 6 fatty acids from cotton collected during first summer trial using FAME and TMS derivatisation methods.

Figure 4. Fatty acid concentrations per sampling days for summer trials: (a) summer 1 – cotton; (b) summer 2 – cotton; (c) summer 1 – polyester; (d) summer 2 – polyester; (e) summer 1 – cotton-polyester; (f) summer 2 - cotton-polyester.

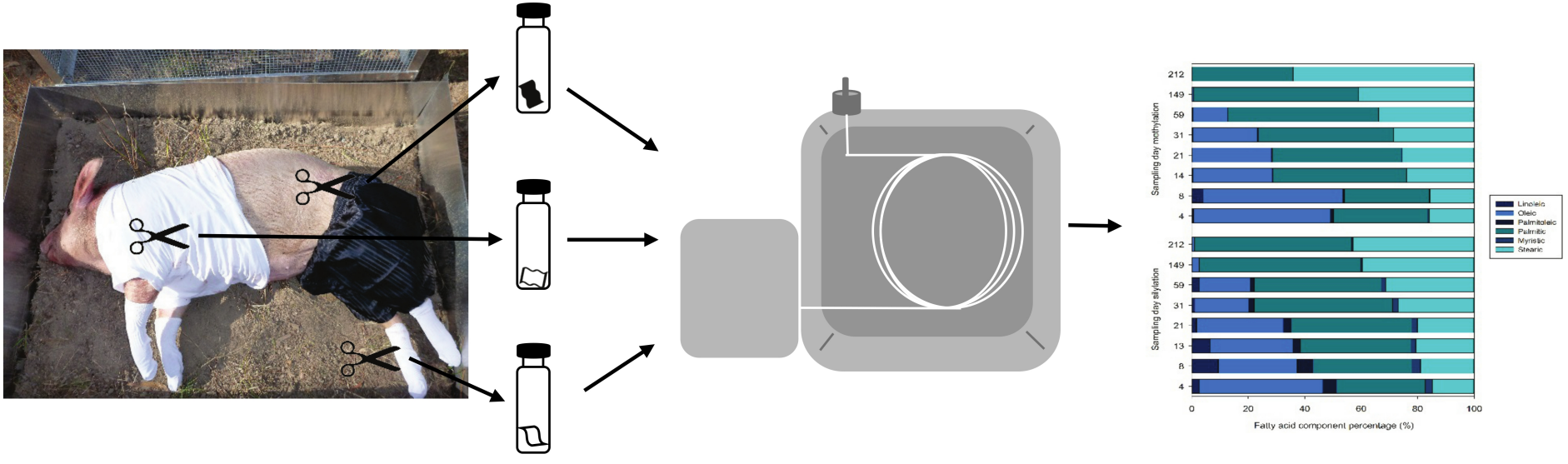
Figure 5. Percentage saturated fatty acids in textiles as a function of surface deposition time: (a) summer 1 – cotton; (b) summer 2 – cotton; (c) winter – cotton; (d) summer 1 – polyester; (e) summer 2 – polyester; (f) winter – polyester; (g) summer 1 - cotton-polyester; (h) summer 2 - cotton-polyester; (i) winter – cotton-polyester.

Figure 6. Fatty acid concentrations per sampling days for winter trial: (a) cotton (b) polyester (c) cotton-polyester.

Figure 7. Oleic and stearic acid concentrations as a function of surface deposition time for summer and winter trials: (a) summer 1 – cotton; (b) summer 2 – cotton; (c) winter – cotton; (d) summer 1 – polyester; (e) summer 2 – polyester; (f) winter – polyester; (g) summer 1 - cotton-polyester; (h) summer 2 - cotton-polyester; (i) winter – cotton-polyester.

Highlights:

- The saturated fatty acid composition showed two different rates of transformation
- The winter trial revealed an initial decrease in saturated fatty acids not detected in the summer trials
- GC-MS could be a useful tool for understanding the behaviour of clothed remains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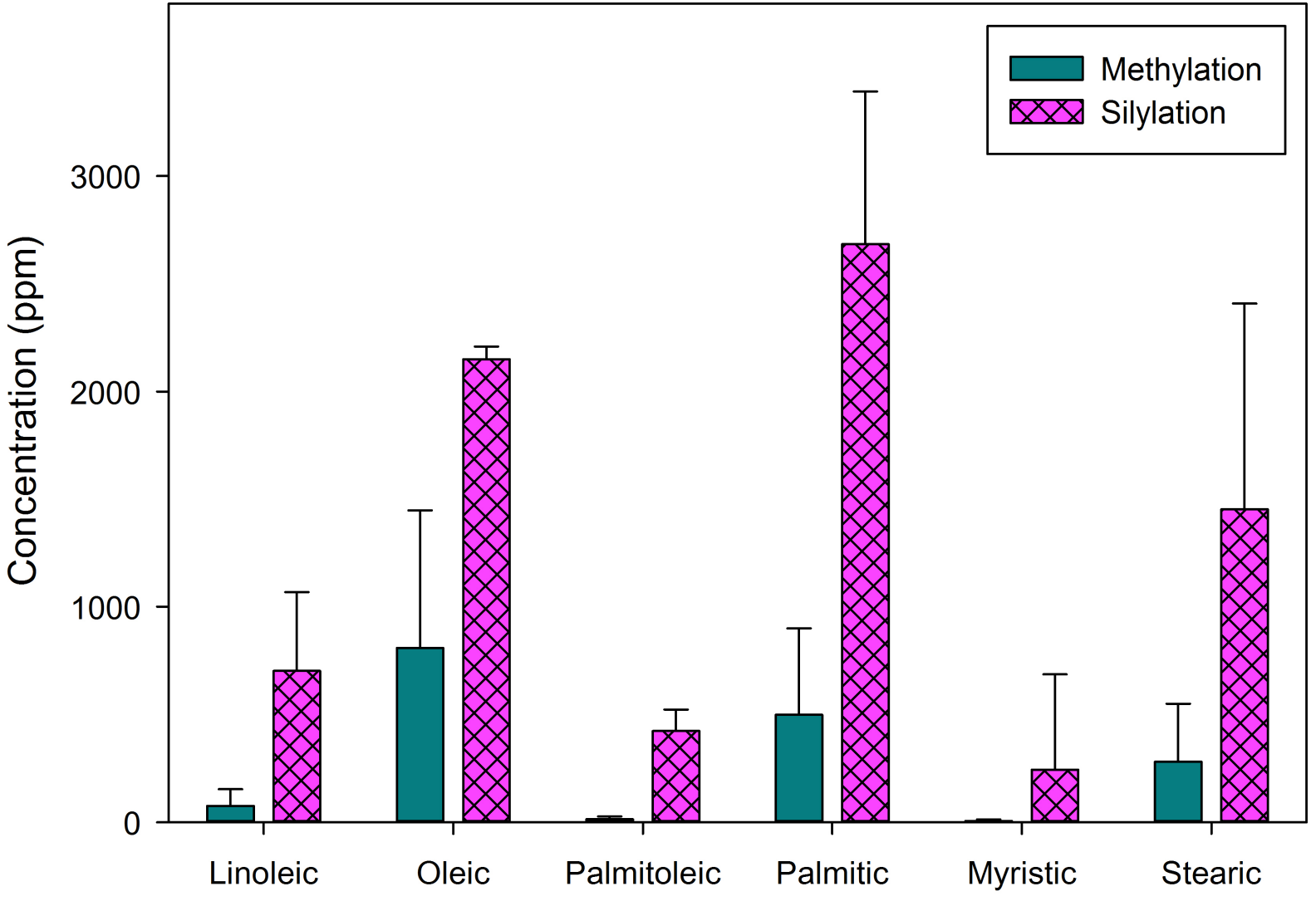
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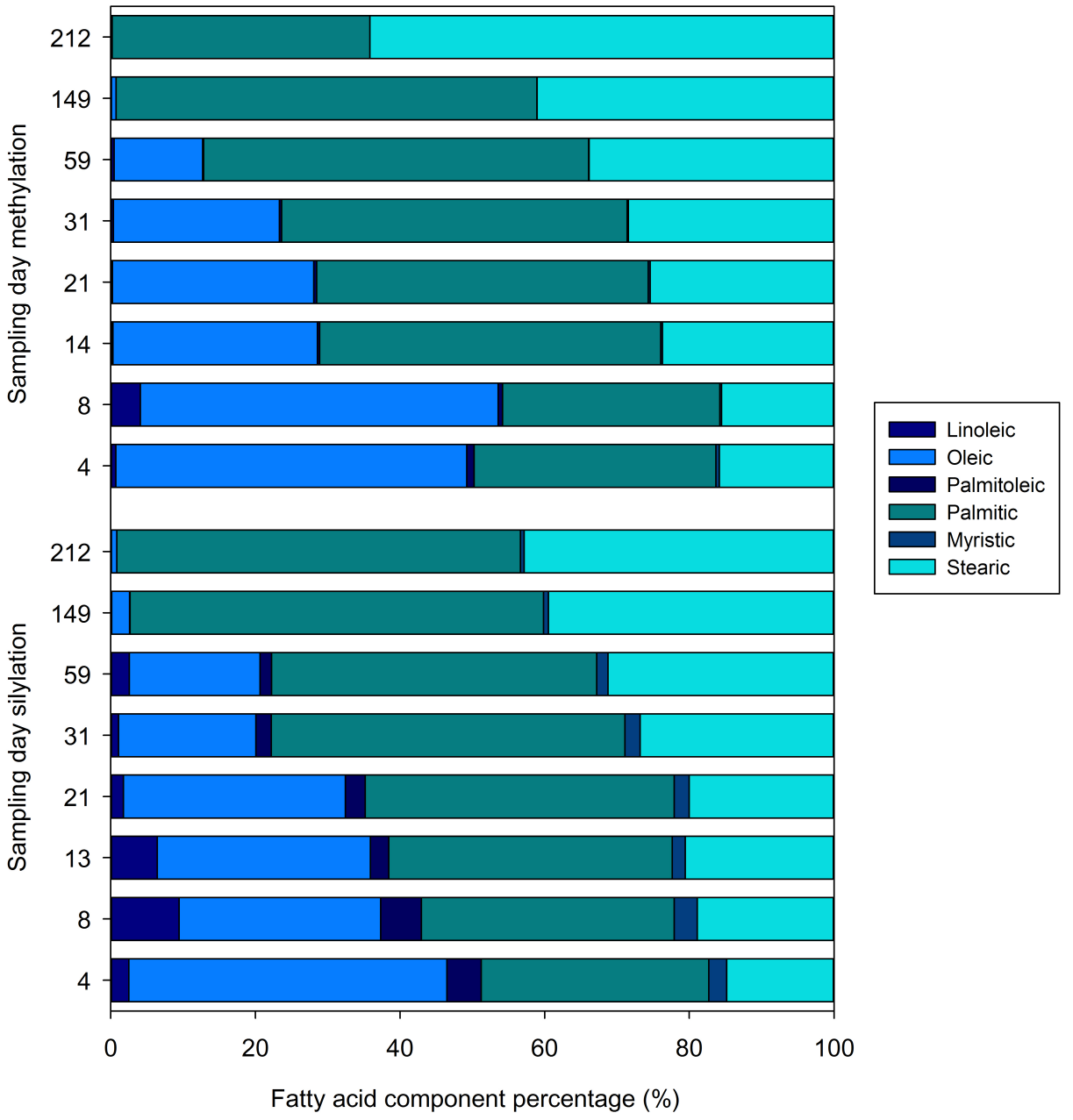
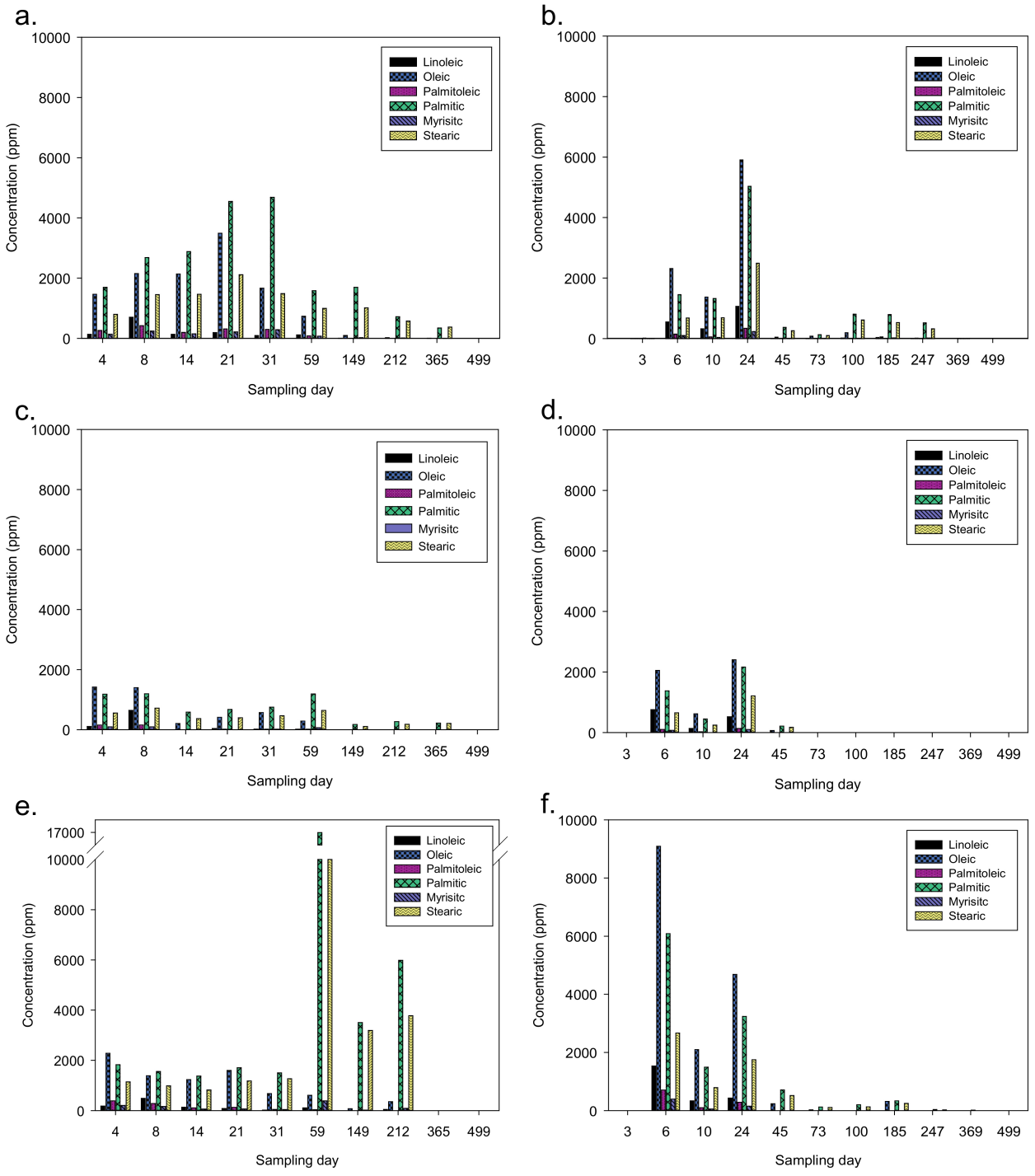
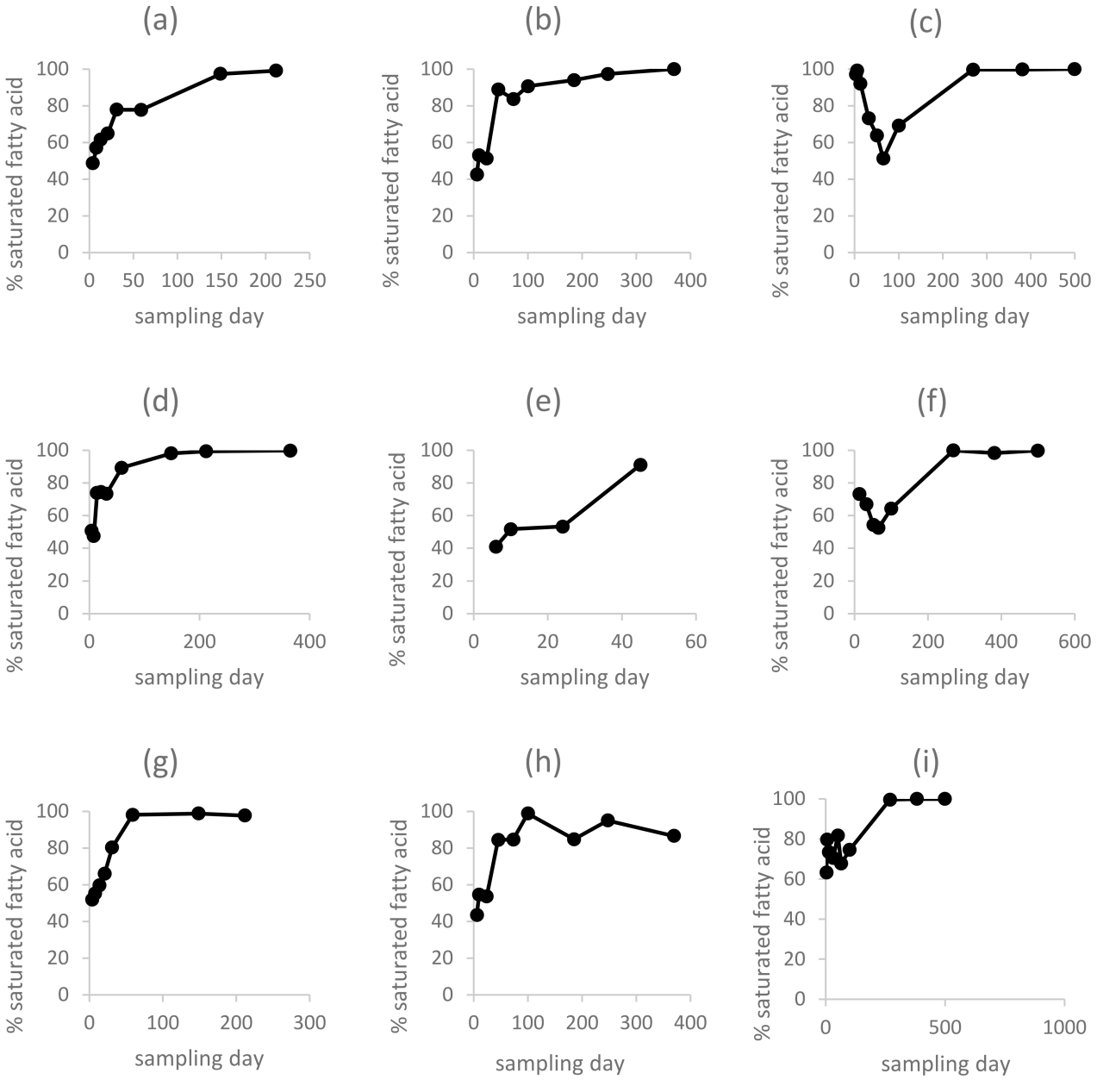
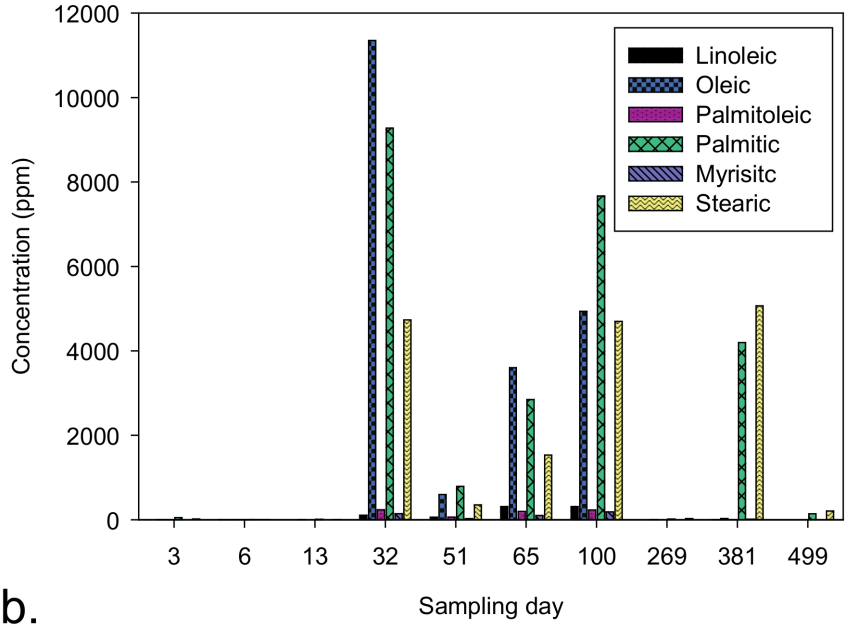
Myristic
Stearic


Sampling day methylation
212
149
59
31
21
14
8
4
Sampling day silylation
212
149
59
31
21
13
8
4
0
20
40
60
80
100
Fatty acid component percentage (%)
Linoleic
Oleic
Palmitoleic
Palmitic
Myristic
Stearic

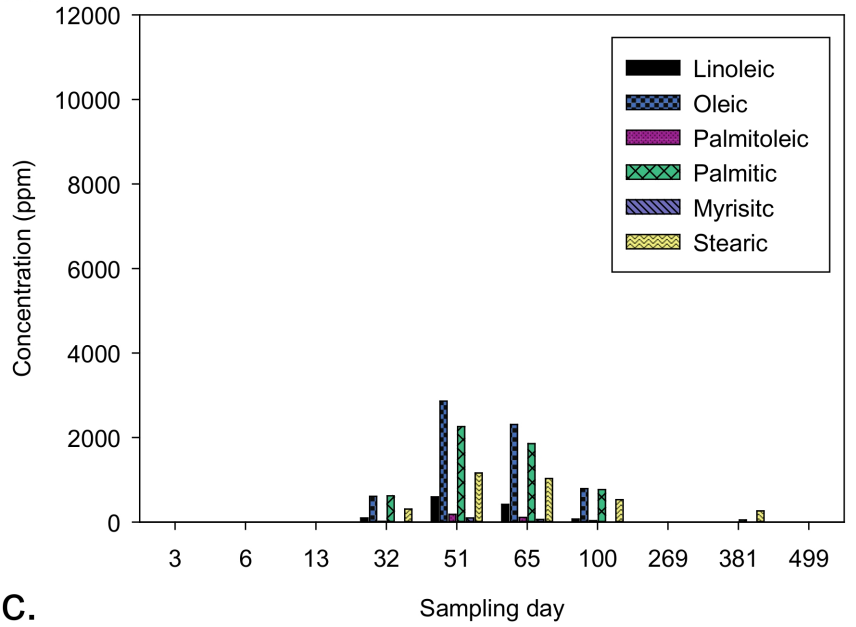

a.
b.
c.
d.
e.
f.
Linoleic
Oleic
Palmitoleic
Palmitic
Myrisitc
Stearic
Concentration (ppm)
Sampling day
0
2000
4000
6000
8000
10000
17000
4
8
14
21
31
59
149
212
365
499
3
6
10
24
45
73
100
185
247
369


(a)
% saturated fatty acid
sampling day
(b)
% saturated fatty acid
sampling day
(c)
% saturated fatty acid
sampling day
(d)
% saturated fatty acid
sampling day
(e)
% saturated fatty acid
sampling day
(f)
% saturated fatty acid
sampling day
(g)
% saturated fatty acid
sampling day
(h)
% saturated fatty acid
sampling day
(i)
% saturated fatty acid
sampling day

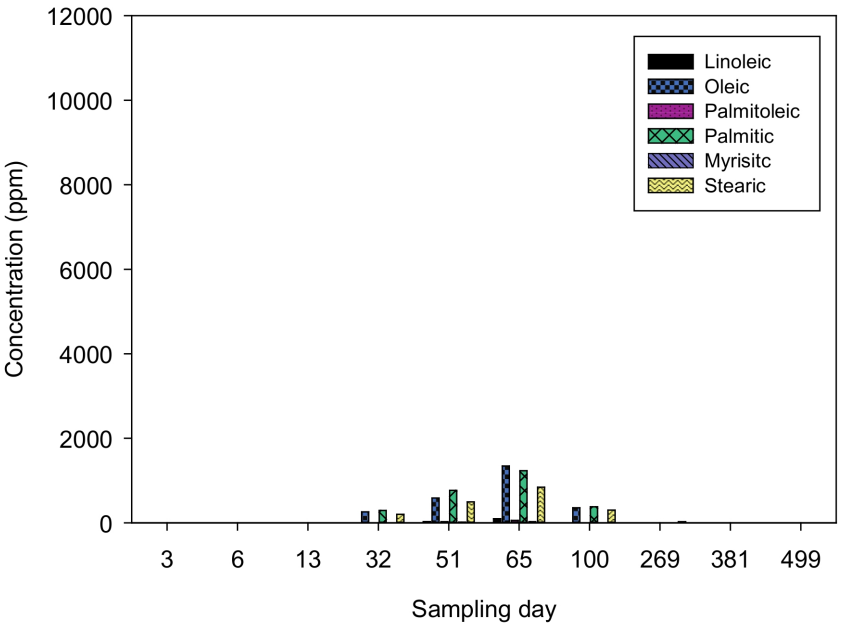
a.

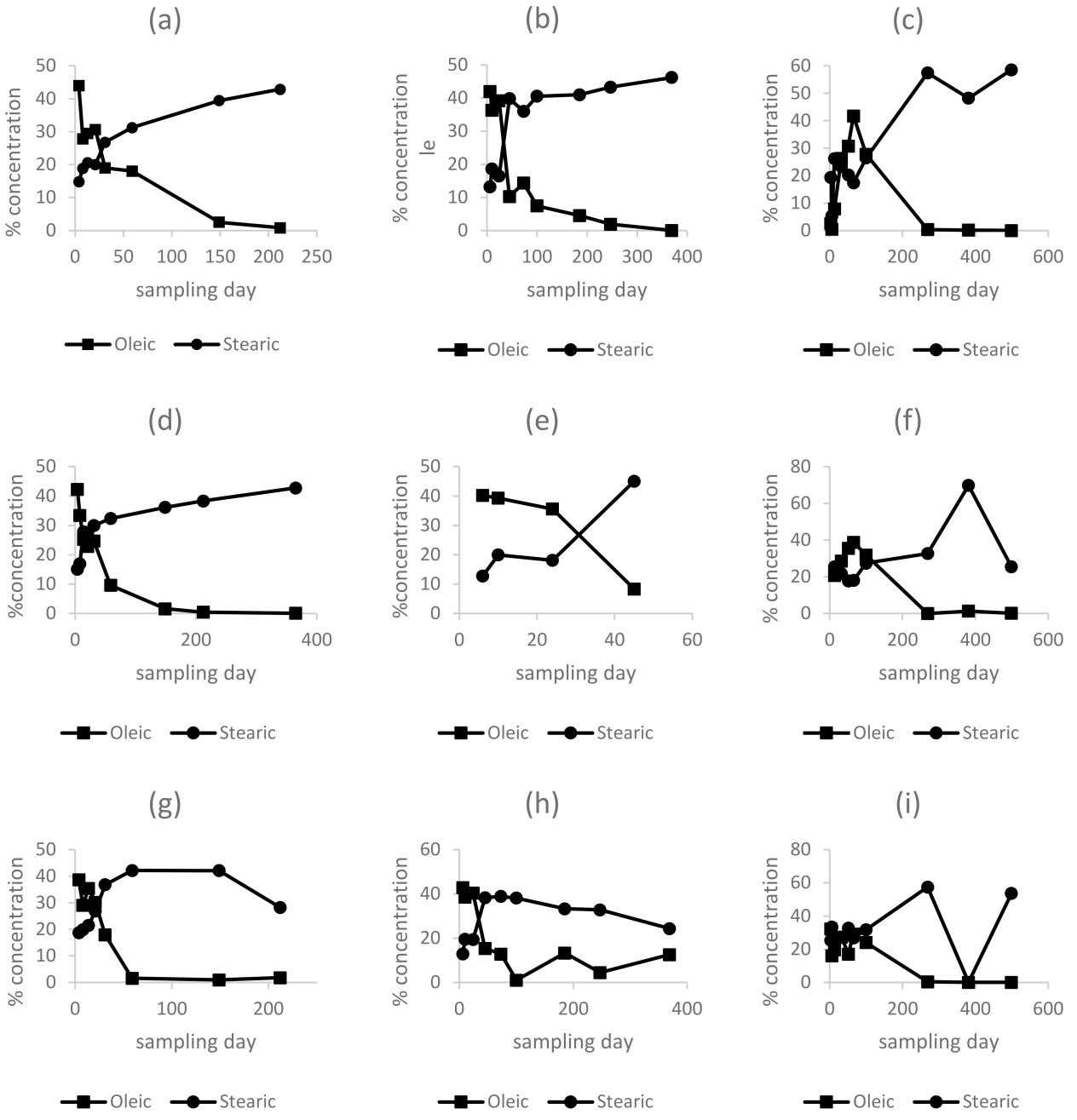

Concentration (ppm)
12000
10000
8000
6000
4000
2000
0
Linoleic
Oleic
Palmitoleic
Palmitic
Myrisitc
Stearic
3
6
13
32
51
65
100
269
381
499
Sampling day

b.


Concentration (ppm)
12000
10000
8000
6000
4000
2000
0
Linoleic
Oleic
Palmitoleic
Palmitic
Myrisitc
Stearic
3
6
13
32
51
65
100
269
381
499
Sampling day

c.


Concentration (ppm)
12000
10000
8000
6000
4000
2000
0
Linoleic
Oleic
Palmitoleic
Palmitic
Myrisitc
Stearic
3
6
13
32
51
65
100
269
381
499
Sampling day


(a)
% concentration
sampling day
Oleic
Stearic
(b)
% concentration
le
sampling day
Oleic
Stearic
(c)
% concentration
sampling day
Oleic
Stearic
(d)
%concentration
sampling day
Oleic
Stearic
(e)
%concentration
sampling day
Oleic
Stearic
(f)
% concentration
sampling day
Oleic
Stearic
(g)
% concentration
sampling day
Oleic
Stearic
(h)
% concentration
sampling day
Oleic
Stearic
(i)
% concentration
sampling day
Oleic
Stearic

The authors have no conflict of interest.