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The effect of the method of burial on adipocere formation

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Abstract

A controlled laboratory experiment was conducted in order to investigate the effect of the method of burial (i.e. the presence of coffin and clothing) on the formation of adipocere. This study follows previous studies by the authors who have investigated the effect of physical conditions on the formation of adipocere present in a controlled burial environment. The study utilises infrared spectroscopy to provide a preliminary lipid profile of the remains following a 12 month decomposition period. Inductively coupled plasma-mass spectrometry was employed as a technique for determining the salts of fatty acids present in adipocere. Gas chromatography–mass spectrometry (GC–MS) was used as the confirmatory test for the identification and determination of the chemical composition of adipocere which formed in the controlled burial environments. The results suggest that coffins will retard the rate at which adipocere forms but that clothing enhances its formation. The results concur with previous observations on adipocere formation in burial environments.
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1. Introduction

When a body is interred in the earth the process of decomposition is ultimately affected by the surrounding environment in which the remains are buried. The burial environment is generally defined as the chemical, biological and geological conditions that prevail in a particular location. Such conditions are modified by the presence of a body and a number of factors will be influential in its decay [1]. The factors affecting decomposition can include the method of burial (e.g. coffin type, clothing type, depth of burial) as well as the physical conditions of the grave site (e.g. soil type, moisture, temperature, air content) [2]. In many burial environments, these conditions will aid decomposition until the body is no more than a skeleton, but under specific circumstances soft tissue preservation may be observed. Two common forms of soft tissue preservation often observed in a burial environment are mummification and adipocere formation [1,3–5].

Mummification results from the desiccation of soft tissues and can affect the entire body or specific regions of the body which are exposed to a desiccatory environment. Hot, dry burial conditions are most favourable to mummification as they allow rapid drying of the soft tissues which prevents putrefaction by destructive micro-organisms [1,4]. The skin becomes dark, dry and leathery and the internal organs

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desiccate and shrink. An entire body can mummify in a matter of days or weeks. After several weeks a body may appear preserved with some shrinkage due to dehydration. Once a body has become mummified it can remain preserved for years [6].

Adipocere formation occurs by the alteration of the soft, fatty tissue of a corpse into a greyish-white paste-like substance [2]. With time this substance will become a hard, brittle shell [5] which acts as a retardant to decomposition. The essential process of adipocere formation results from the hydrolysis and hydrogenation of adipose fats [7]. Extensive studies regarding its chemical composition demonstrate that it consists predominantly of saturated fatty acids, namely myristic, palmitic and stearic acids, with lesser amounts of hydroxy and oxo-fatty acids [8–12]. The formation of adipocere is considered to result from bacterial action and is favoured by anaerobic environments whereby sufficient moisture is present in the tissue [7,9]. The rate of formation can take weeks to months [13] and is largely dependent on the surrounding environment. Once formed, it may be retained for hundreds [14] and even thousands of years [15–17]. However, adipocere is not an end product and decomposition of adipocere may occur under certain aerobic [2] and bacterial conditions [11].

The occurrence of adipocere in grave sites is well documented [3,11,18] and in some countries creates problems for cemeteries by preventing the re-use of graves and limiting the burial space available [2]. A burial environment will often contain a number of factors which inhibit bacterial growth, thus retarding decomposition and setting up favourable preservation conditions. The nature of the soil and sediments has been investigated [1,3] and its effect on adipocere formation reported previously by the authors [19]. The effect of the physical conditions of a burial environment (e.g. soil pH, temperature, moisture and oxygen content) on the formation of adipocere has also been reported [20].

The aim of the current study was to investigate the effect of the method of burial, namely coffin environment and clothing, on adipocere formation. Observations by Mant [3,8] suggest that bodies decompose more rapidly in coffins when compared to bodies buried directly in soil. Burial in coffins accelerates the post-mortem dissolution of the body and would likely yield an unfavourable environment for adipocere formation. When adipocere was observed in coffin burials, it was often scanty, appearing mainly on the thighs [3].

Many of the bodies on which Mant based his observations were buried without coffins and were fully clothed. Adipocere formation was generally found to be more advanced where clothing was present, even in shallow burials [8]. This was most likely due to the ability of the clothing to absorb and retain moisture, thus aiding the formation of adipocere [3]. Studies by Mellen et al. [13] and Manhein [21] concur with Mant's observations on the effect of clothing.

The focus of this study was to visually observe adipocere formation in a controlled environment and investigate its chemical composition to determine whether the extent of formation could be determined quantitatively. Infrared spectroscopy was used to provide a preliminary lipid profile of the adipocere samples, as well as for identifying fatty acid salts. Inductively coupled plasma–mass spectrometry (ICP–MS) provided more information regarding the presence of salts of fatty acids within the adipocere by identifying the major cations. Additionally, gas chromatography–mass spectrometry (GC–MS) was employed as the method for the identification of the major components of adipocere. The results are useful in suggesting the possible effect of the method of burial on the rate and extent of adipocere formation.

2. Materials and methods

2.1. Adipocere formation

Whilst soil and adipocere samples collected from grave exhumations have previously been analysed by the authors [12,18,22], this study required the decomposition and formation of adipocere to occur in a controlled environment so that the individual variables could be assessed. Burial environment microcosms were prepared in large decomposition containers which involved a tap for draining purposes and an airlock seal for the release of gases as decomposition proceeded. Due to ethical restrictions within Australia, the use of human adipose tissue was not an option for this study. Current considerations suggest that pig cadavers best mimic human decomposition [23,24] and this study utilised domestic pigs which were reared on identical diets for commercial use. A section of pig adipose tissue collected from the abdominal region and still containing muscle and skin was used to prepare each experiment. To ensure adipocere formation, each environment was prepared using those factors known to result in adipocere formation, namely sufficient adipose tissue, moisture, bacteria, and a relatively anaerobic environment [3,14]. A control environment was created, using a damp soil consisting of a loamy sand composition, in a temperate climate which averaged approximately 22 °C over a 12 month period. Additional experiments were prepared in the same manner with the individual variable of interest altered accordingly.

Control samples were prepared by burying the fatty tissue directly in the soil. To investigate the effect of coffin burials on adipocere formation, mock coffins were prepared from particle chipboard. The coffins were lined with plastic sheeting obtained from a funeral director in Sydney, Australia. A layer of satin material was placed over the plastic lining, and the pig tissue placed inside the mock coffin. The lid, which was also lined with plastic and material, was nailed shut. Unlined coffins were also prepared whereby the fatty tissue was placed directly into the mock coffin so that

the tissue was in contact with the wood. It was of interest in this study to determine whether the contact of tissue with wood had any affect on adipocere formation. Both the lined and unlined coffins were buried in moist soil, and the decomposition containers sealed tightly to produce an anaerobic environment.

Samples were also buried in plastic bags in an attempt to mimic a forensic situation in which remains are disposed of in bags in order to avoid immediate detection. A report by Manhein [21] concluded that a large percentage of bodies which formed adipocere had coverings of some kind, including plastic. Plastic bags were used as another form of encasing the decomposing tissue in this study. The tissue samples were wrapped in a single layer of low density polyethylene before being buried in moist soil. The decomposition containers were sealed tightly to produce an internal anaerobic environment.

Clothing types were also of interest and the materials used in this part of the study were chosen based on common types of material presently used as clothing: namely, polyester and cotton. Adipose tissue samples were wrapped in a single layer of either cotton or polyester clothing so that the material completely covered the tissue. The wrapped tissue was buried in moist soil in an anaerobic environment.

2.2. Sample collection

Three replicates of the control and of each burial environment were prepared. The experiments were conducted for a period of 12 months. Each experiment was monitored regularly to ensure it accurately mimicked the particular environment which it aimed to investigate. Upon completion of the study the adipocere formation was documented in situ. Samples of adipocere or decomposed tissue were collected from each burial environment and placed in a sealed specimen container prior to analysis.

2.3. Infrared spectroscopy

For each sample approximately 2 mg of adipocere was ground with approximately 10 mg of powdered KBr using a mortar and pestle. The background spectra were recorded using approximately 20 mg of powdered KBr. Each sample was placed in a Spectra-Tech 3 mm microsampling cup for analysis. The samples were investigated using a Nicolet Magna-IR 760 Fourier transform spectrometer equipped with a DTGS detector. The spectra were scanned over the range 4000–500 cm^{-1} and for each sample, 64 scans were recorded with a resolution of 4 cm^{-1}.

2.4. ICP–MS

Five milligram of adipocere was accurately weighed into a plastic screw-top tube and 300 μl each of concentrated HNO_3 (BDH Laboratory Supplies, England), concentrated HCl (Riedel-de Haen, Germany) and H_2O_2 (BDH) added. Each sample was heated until dissolved and allowed to cool. Upon cooling each sample was diluted to 10 ml using distilled water. The analysis was carried out using a Perkin-Elmer SCIEX ELAN 5100 inductively coupled plasma–mass spectrometer featuring a Plasmalok plasma-mass spectrometer interface and a Perkin-Elmer FIAS-400 flow injection system. All samples were injected using a Perkin-Elmer AS-90 autosampler and the data analysed using ELAN software.

2.5. GC–MS

Two milligram of adipocere sample was accurately weighed into a sterilised reacti-vial. One milliliter of chloroform was added and the mixture sonicated for 15 min. The chloroform layer was drawn off and placed in a sterilised screw top tube. Two hundred fifty microliter of hexamethyldisilazane (HMDS) (Sigma–Aldrich, Australia) was added to form the trimethylsilyl esters of fatty acids and the tube heated at 70 °C for 15 min. Upon cooling, an aliquot was removed and placed in a vial for analysis by GC–MS.

All analyses were performed on a Hewlett Packard 5890 Series II gas chromatograph coupled with a Hewlett Packard 5970B Series mass selective detector. A 1 μl aliquot of the sample was injected into a DB5-MS (J&W Scientific, USA) fused-silica capillary column (30 m × 250 mm × 0.25 μm, 5% phenylmethylpolysiloxane). The initial column temperature was 100 °C and the initial time was 1 min. The temperature was increased at 7 °C min^{-1} to 275 °C where it was held for 5 min. All injections were in the splitless mode using a HP 7673 autosampler and injector. The analysis was conducted in a selected ion monitoring mode, and identified those fatty acids known to comprise adipocere. The saturated fatty acids considered were myristic, palmitic, stearic and 10-hydroxy stearic acids. The unsaturated fatty acids, palmitoleic and oleic acids, were also considered because of their occasional presence in low concentrations. Peaks relating to the trimethylsilyl esters of the fatty acids were identified by comparison of their retention time and mass spectra against the NIST98 Mass Spectral Library. The chromatograms obtained for each sample were compared with a standard solution of fatty acid mixtures consisting of myristic, palmitic, stearic and oleic acids [12].

3. Results

3.1. Adipocere collection

At the completion of the 12 month period, the decomposition containers were unsealed and the resulting tissue was documented in situ. The control soil environment was successful in converting the entire mass of tissue into adipocere. The other burial environments demonstrated varying stages of decomposition and adipocere formation.

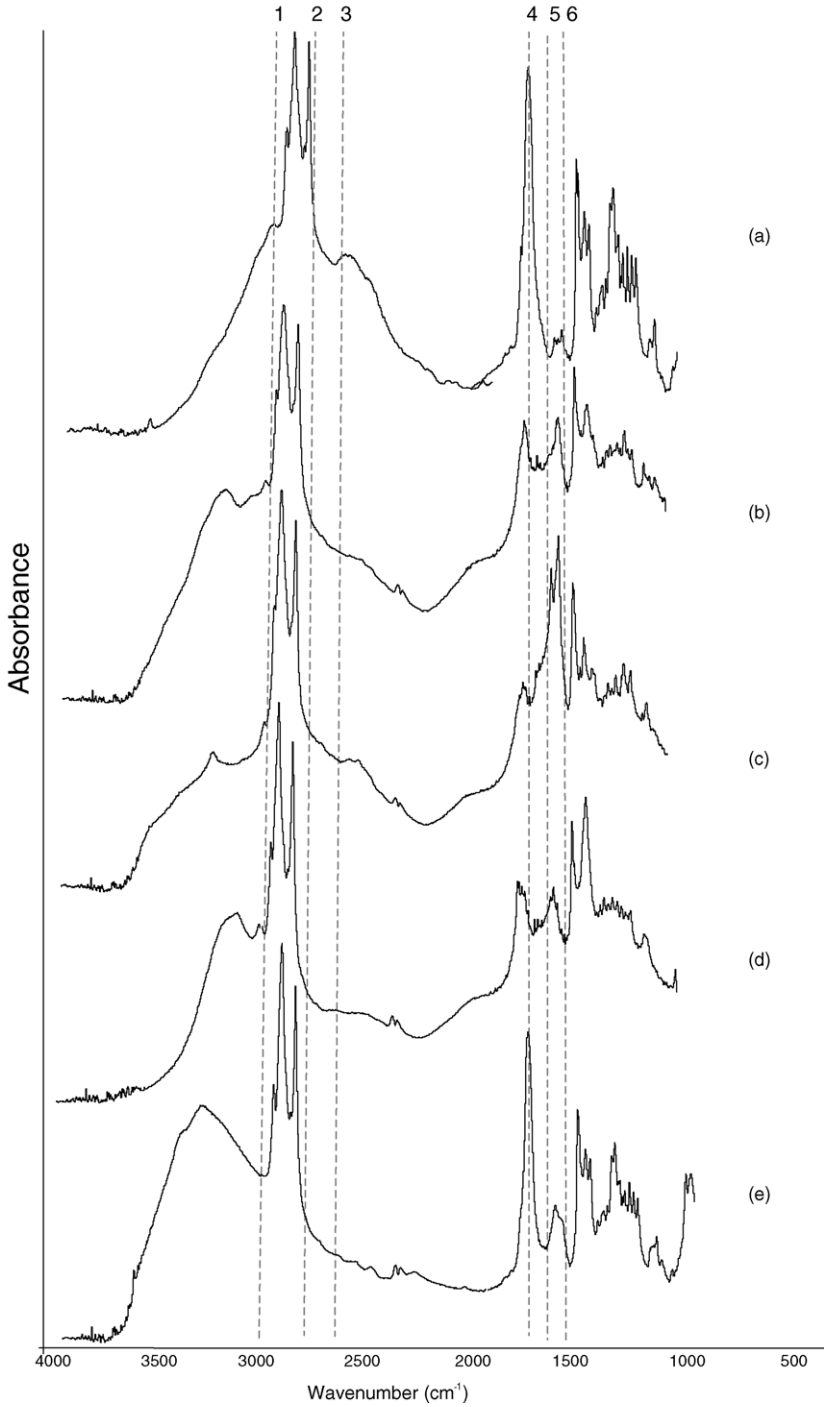

Fig. 1. Characteristic infrared spectra of tissue samples collected from (a) control, (b) lined coffin, (c) unlined coffin, (d) plastic bag and (e) polyester clothing burial environments. Dotted lines represent (1–2) C–H stretching (2950–2800 cm^{-1} region), (3) fatty acid O–H stretching (≈2670 cm^{-1}), (4) fatty acid C=O stretching (≈1700 cm^{-1}), (5–6) fatty acid carboxylate C–O stretching (1576–1540 cm^{-1} region).

Although the wood had not disintegrated, the mock coffins were no longer well sealed at the completion of the experiment. Upon opening the coffins, a white fungus layer was observed covering the entire surface of the decomposed tissue. The fungus had the appearance of cotton wool, and was sampled for analysis. The remaining tissue was a grey-black colour with small amounts of skin still intact. A scanty white solid substance was also observed amongst the decomposed tissue.

The samples buried in plastic bags produced an unusual result. Based on previous studies [21] it was expected that a plastic covering would aid adipocere formation. However, examination of the bags showed that the remains had liquefied but had not formed a solid substance characteristic of adipocere. The decomposed remains were in a semi-liquid state demonstrating a viscous texture. Samples of the liquefied remains were collected for analysis.

Examination of the polyester clothing environment showed the polyester material was still intact and appeared to retain a quantity of moisture from the decomposition process. Observation of the remains demonstrated good preservation of the tissue and a white solid substance characteristic of adipocere. The cotton clothing environment, however, produced an unexpected result. During the 12 month period, the cotton clothing had completely disintegrated and the tissue sample had decomposed so that no visible remains were observed in these environments. Consequently, the cotton clothing experiments were discarded from the study, although suggestions as to the possible cause of the complete decomposition will be outlined in Section 4.

3.2. Fungi analysis

The white, cotton wool-like fungus layer observed in the mock coffin environments was analysed by a microbiologist for identification purposes. The layer of fungus which covered the decomposed remains was found to contain four fungi species.

The first was a *Chrysosporium species* in the Phylum Ascomycota. Ascomycota accounts for approximately 75% of all fungi described, including the fungi that lack morphological evidence of sexual reproduction. Due to the lack of sexual reproductive structures in the culture, the organism, in this instance, could not be identified to a species level. It was however, determined to be saprophytic, confirming that it thrived on dead organic matter.

The second fungus was a *Penicillium species* also in the Phylum Ascomycota and described as ubiquitous. The third fungus was a *Mucor species* which is classed in the Phylum Zygomycetes. Also described as ubiquitous, *Mucor* is a filamentous fungus commonly found in soil, plants and decaying matter. The fourth fungus was difficult to identify and may have been a *Dermatophyte species* in the Phylum Ascomycota. The most common types are *Trichophyton* or *Microsporum*, both being pathogens to humans and animals. *Dermatophyte* fungi are more commonly described as ringworm fungi and depend on their host (e.g. animal skin) to survive.

3.3. Infrared spectroscopy

Infrared bands attributable to adipocere have been previously identified using infrared spectroscopy [15,22] and include triglycerides ($\sim$1740 cm^{-1}), fatty acids ($\sim$1700 cm^{-1}), and salts of fatty acids ($\sim$1576–1540 cm^{-1}). Fig. 1a illustrates the infrared spectrum characteristic of the adipocere collected from the control burial environment. The control samples contain a high concentration of saturated fatty acids as indicated by the band at 1700 cm^{-1}. A broad band representative of hydroxy fatty acids is observed in the region 2680–2670 cm^{-1}, whilst small amounts of salts of fatty acids are indicated by the presence of bands in the region 1576–1540 cm^{-1}. Strong C–H stretching bands are visible in the region 2950–2800 cm^{-1} and a small shoulder attributable to =C–H stretching of unsaturated fatty acids can be seen in the higher wavenumber end of this region. The preliminary lipid profile indicates the formation of adipocere.

A characteristic infrared spectrum of the samples collected from the lined coffin environment is shown in Fig. 1b. The spectrum shows a moderate band relating to triglycerides at 1735 cm^{-1} and a weak band indicative of unsaturated fatty acids at 1652 cm^{-1}. The spectrum lacks bands relating to salts of fatty acids. C–H stretching bands are visible in the region 2950–2800 cm^{-1} whilst a weak band in the high wavenumber end of this region is also suggestive of unsaturated fatty acids. The preliminary lipid profile, as demonstrated by infrared spectroscopy, suggests that the original adipose tissue has undergone decomposition as evidenced by the reduced triglyceride peak. The process of adipocere formation may have commenced although the characteristic fatty acid band ($\sim$1700 cm^{-1}) is not visible.

The characteristic infrared spectrum of the unlined coffin environment, illustrated in Fig. 1c, is similar to the spectrum representing the lined coffin environment. The spectrum contains a small band at 2658 cm^{-1} which is evidence of hydroxy fatty acids, but lacks a visible band at wavenumber 1700 cm^{-1}. The weak bands at 1736 and 1652 cm^{-1} are evidence of the presence of triglycerides and unsaturated fatty acids, respectively. Moderate bands are observed at 1576 and 1540 cm^{-1}, indicating the presence of salts of fatty acids. The identification of fatty acid salts is the major difference observed between the spectra of the lined and unlined mock coffin environments.

Fig. 1d is the characteristic infrared spectrum of the viscous samples collected from the plastic bag burial environments. The spectrum contains a moderate band at 1736 cm^{-1}, indicative of triglycerides remaining in the samples. Moderate bands in the region 3110–3000 cm^{-1}, combined with a weak band at 1662 cm^{-1}, are evidence of the presence of unsaturated fatty acids. The spectrum lacks

Table 1
Average major cation composition of samples using ICP–MS

Burial type	Relative abundance as a proportion of total cations determined			
	Na	K	Ca	Mg
Control	0.01	0.09	0.75	0.15
Lined coffin	0.07	0.89	0.02	0.02
Unlined coffin	0.03	0.71	0.10	0.16
Plastic bag	0.11	0.68	0.04	0.17
Polyester clothing	0.00	0.01	0.51	0.48

bands relating to salts of fatty acids. The profile suggests that rapid decomposition occurred in the plastic bag environment which is consistent with the liquefied remains observed.

The final spectrum detailed in Fig. 1e demonstrates a characteristic profile of the samples collected from the polyester clothing environment. A strong band representative of fatty acids is observed at 1700 cm^{-1}, whilst smaller bands in the region 1580–1540 cm^{-1} are indicative of salts of fatty acids. The spectrum lacks bands attributable to triglycerides and unsaturated fatty acids suggesting that adipocere formation occurred in the burial environment containing polyester clothing.

3.4. ICP–MS

Inductively coupled plasma–mass spectrometry is a useful technique for detecting the abundance of cations in adipocere which aids in the identification of salts of fatty acids. The salts which are generally considered to be present in adipocere are sodium, potassium, calcium, and magnesium salts of fatty acids [25,26]. For this study, the absolute abundance of each cation was calculated as a concentration in ppm. The values were converted to a relative abundance value (as a proportion of the total cations of interest) and an average calculated for each burial environment. Table 1 lists the average composition of the major cations detected in the samples collected from the burial environments.

ICP–MS analysis of the adipocere samples collected from the control burial environment demonstrated a high abundance of calcium ions. A study by Gill-King [25] suggests that fatty acid chains, which are released during the decomposition process, take up sodium and potassium from the internal environment. When a cadaver is placed in soil, the sodium and potassium ions are replaced by calcium [25] and magnesium [26] ions which are present in the external environment. The high abundance of calcium ions identified in the control samples is likely due to cation exchange between the decomposing tissue and the surrounding soil environment.

The samples obtained from the mock coffin environments show a high abundance of potassium ions and a small abundance of sodium, calcium, and magnesium ions. The mock coffin environments and control environments both consisted of the same soil type, yet demonstrate a considerable difference in cation composition. The difference may be attributed to the barrier provided by the mock coffins between the decomposing tissue and surrounding soil. The abundance of potassium ions is likely a reflection of the saponification which occurred early in the decomposition process. As the tissue was not in contact with the soil during decomposition, it would not have had an opportunity to undergo cation exchange, thus replacing the potassium ions with calcium or magnesium ions.

The viscous samples collected from the plastic bag burial environment also demonstrated an abundance of potassium ions with lesser abundances of sodium, calcium and magnesium ions. The result can most likely be described using the same explanation as the mock coffin burial environments. As the plastic bag was non-biodegradable, the decomposing remains did not come into contact with the surrounding soil in which it was buried. The plastic bag provided a barrier between the decomposing tissue and surrounding soil environment, thus inhibiting cation exchange. The abundance of potassium salts of fatty acids in the samples is therefore a result of the saponification which occurred between the fatty acids and potassium ions released during decomposition.

The analysis of the samples buried in polyester clothing demonstrated high abundances of calcium and magnesium ions, but low abundances of sodium and potassium ions. Although the tissue was wrapped in polyester clothing and was not in direct contact with the soil, decomposition fluids were able to permeate through the material as was observed at the completion of the experiment. It is likely that cation exchange between the decomposing tissue and surrounding soil occurred, thus reducing the relative abundance of sodium and potassium ions, and further increasing the relative abundance of calcium and magnesium ions. The majority of salts identified in the clothed samples comprised of calcium and magnesium salts of fatty acids.

3.5. GC–MS

A previous study [27] by one of the authors of a large quantity of adipocere samples demonstrated a ‘standard’ ratio of fatty acids which was indicative of adipocere formation. In all of the samples which demonstrated well-formed adipocere, palmitic acid accounted for approximately half the concentration of fatty acids. Stearic acid was the next dominant of the fatty acids usually accounting for approximately a third of the fatty acids. The concentrations of myristic and 10-hydroxystearic acid varied but each represented less than 10% of the chemical composition. In many instances, 10-hydroxystearic acid was not detected in the adipocere samples. The only unsaturated fatty acid which was regularly detected was oleic acid and normally accounted for less than 5% of the fatty acid concentrations.

Table 2 lists the relative percentage composition of the fatty acids identified in the samples collected from the various burial environments in this study. The fatty acid

Table 2
Relative percentage (%) composition of fatty acids detected using GC–MS

	Saturated fatty acids				Unsaturated fatty acids	
	Myristic $C_{14:0}$	Palmitic $C_{16:0}$	Stearic $C_{18:0}$	10-Hydroxy stearic	Palmitoleic $C_{16:1}$	Oleic $C_{18:1}$
Control burial	5.1	58.7	29.2	1.1	0.0	6.0
Lined coffin	1.6	42.0	24.9	10.1	0.4	21.0
Unlined coffin	1.7	45.8	24.6	11.7	0.8	15.5
Plastic bag	2.7	36.1	21.3	0.8	3.3	34.9
polyester clothing	4.4	61.0	32.0	0.0	0.0	2.6

value represents an average of three repeat measurements for each of the replicate samples collected from the individual burial environments. The control samples demonstrate a composition which is high in saturated fatty acids and low in unsaturated fatty acids. The dominant saturated fatty acids are palmitic acid, followed by stearic acid. Myristic acid and 10-hydroxy stearic acid are also identified in smaller quantities. Approximately 94% of the relative composition is comprised of saturated fatty acids. The chemical composition of the samples collected from the control burial environment is characteristic of adipocere [12], although the adipocere product is not chemically stable.

The samples collected from the mock coffin environments (both lined and unlined) show very similar chemical compositions. The dominant fatty acids are palmitic and stearic acid, although the relative percentage of palmitic acid is reduced when compared to the control. Additionally, an increased percentage of 10-hydroxy stearic acid and oleic acid is observed for the mock coffin environments. The percentage of unsaturated fatty acids is higher (approximately 16–21%) and the percentage of saturated fatty acids is lower (approximately 79–84%) than in the control samples. The relative percentage composition for the samples suggests that the chemical conversion to adipocere has taken place, but the extent to which it proceeded was retarded by the mock coffin environments.

The viscous samples collected from within the plastic bag environment have a chemical composition which is much higher in unsaturated fatty acids than the other samples in this study. Although not listed in Table 2, the samples contained a small percentage of linoleic acid resulting in a total unsaturated fatty acid content of almost 40%. The total saturated fatty acid content was approximately 61% which comprised a notable reduction in the percentage of palmitic acid. The chemical composition is comparable to the composition of adipose tissue [28] suggesting that the samples represent a liquefied form of the original tissue. The chemical composition is not characteristic of adipocere and it appears that adipocere formation was unable to occur in the plastic bag burial environment.

The samples collected from the polyester clothing environment had a total relative composition of saturated fatty acids of more than 97%. The only unsaturated fatty acid remaining in the samples is oleic acid, which is present at a considerably low level. Although 10-hydroxy stearic acid is absent from the samples, the chemical composition is indicative of adipocere. The polyester clothing burial environment appears to be conducive to adipocere formation, possibly more so than the control environment without clothing.

4. Discussion

The control burial environment used in this study comprised those factors which are considered to induce adipocere formation; fatty tissue, moisture, bacteria, and an anaerobic environment. These factors were successful in forming adipocere in a loamy, sand soil environment. All the other burial environments contained the same basic burial factors and were therefore able to be compared with the control environment to determine the extent of adipocere formation.

It was of interest in this study to determine whether the lining of a coffin had any affect on adipocere formation. Subsequently, some coffin burial environments were investigated containing plastic and material lining whilst others were without. At the completion of the experiment, both coffin environments contained a white fungus across the surface of the decomposed remains, and demonstrated similar degrees of decomposition with what appeared to be scanty adipocere formation. The results of the infrared spectroscopy, ICP–MS and GC–MS analyses were similar for all coffin environments suggesting that the presence or absence of a plastic or material lining does not have an obvious affect on the chemical conversion to adipocere.

The mock coffin environments did however, appear to retard the rate at which adipocere formed when compared to the control environment. The presence of small quantities of triglycerides coupled with higher percentages of unsaturated fatty acids, suggests that the process of conversion to adipocere occurred at a slower rate in the mock coffin environment, when considering all other factors to be equal. The adipocere which formed in this study was scanty and the majority of the tissue sample simply decomposed. The result concurs with a study by Mant [3] in which he notes that bodies buried in coffins decomposed more rapidly than those buried directly in soil. The more rapid decomposition is

presumably due to the slightly aerobic environment within the coffin which consequently inhibits the formation of adipocere. A slightly aerobic environment was present within the mock coffins of this study as evidenced by the formation of aerobic fungi.

At the conclusion of the experiment it was evident that the plastic bag environment did not induce adipocere formation as demonstrated by the liquefied tissue which remained. Decomposition of the original tissue occurred resulting in a disintegrated semi-liquid mass. The non-biodegradable nature and lack of diffusion properties associated with the plastic bag meant that the semi-fluid mass was excluded from leaching into, or interacting with, the surrounding soil environment. The chemical analysis of the viscous samples confirmed that decomposition had occurred, as evidenced by the presence of triglycerides and unsaturated fatty acids, and that the composition was chemically similar to that of the original adipose tissue.

Overall, the lack of adipocere in the plastic bag experiment was unexpected, as previous studies [3,21,29] had suggested that coverings, including plastic, have a profound effect on adipocere formation. It is however worth noting that when the liquefied remains were firstly wrapped in clothing and then in a plastic bag, the mass rapidly solidified and adipocere formation was observed within a few months. Unfortunately, the result was only based on observation and a chemical analysis was unable to be conducted for the clothing/plastic bag burial environment. It is reasoned that the lack of adipocere formation within the plastic bag environment is a result of the inability of plastic to absorb the decomposed fluids. When clothing was placed within the plastic bag environment, it was able to act as an absorbent, thereby removing the decomposition by-products (e.g. glycerol) which prohibit adipocere formation.

The lack of adipocere formation in the cotton clothing burial environment was also unexpected, as the presence of clothing is considered to be conducive to adipocere formation [3,13]. Studies conducted by Morse and Dailey [30] investigated the preservation of various clothing fibres buried in soil. Their results showed that cotton materials had a life expectancy of less than 10 months due to their organic nature. The suggested life expectancy correlates with the results identified in this study which demonstrate that the cotton clothing had completely disintegrated after 12 months burial duration. It is unclear why the tissue decomposed rapidly following the disintegration of the cotton clothing but it may be due to the bacteria present in the soil environment and their association with the cotton clothing.

In contrast, the polyester clothing was still well preserved after 12 months burial duration, a phenomenon that has been observed in the field [31]. The polyester clothing was conducive to adipocere formation as demonstrated by the high saturated fatty acid composition and lack of triglycerides remaining in the samples. Polyester clothing has the ability to retain moisture which may have aided the formation of adipocere. The polyester also allowed cation exchange by diffusion of the decomposition fluids through the material thus causing saponification and 'hardening' of the adipocere [25]. The results concur with observations by Mant [3] and Mellen [13] which propose that adipocere formation is generally more extensive in bodies covered by clothing.

5. Conclusion

This study was conducted to determine the effect of the method of burial on adipocere formation in a controlled burial environment. The results confirm previous observations that adipocere forms more readily on a body which is buried directly in soil, as opposed to a body buried in a coffin. Plastic coverings were associated with the liquefaction of the fatty tissue and inhibit adipocere formation. However, tissue which was wrapped in clothing and plastic, or polyester clothing, was identified as being conducive to adipocere formation. The results are valuable because they indicate the likely effect/s of particular factors associated with the method of burial on the formation of adipocere in a soil environment. The conclusions are based on both observation and chemical analyses of the decomposed remains.

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