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The effect of the burial environment on adipocere formation

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Abstract

Adipocere is a decomposition product comprising predominantly of saturated fatty acids which results from the hydrolysis and hydrogenation of neutral fats in the body. Adipocere formation may occur in various decomposition environments but is chiefly dependent on the surrounding conditions. In a soil burial environment these conditions may include such factors as soil pH, temperature, moisture and the oxygen content within the grave site. This study was conducted to investigate the effect of these particular burial factors on the rate and extent of adipocere formation. Controlled laboratory experiments were conducted in an attempt to form adipocere from pig adipose tissue in model burial environments. Infrared spectroscopy, inductively coupled plasma–mass spectrometry, and gas chromatography–mass spectrometry were employed to determine the lipid profile and fatty acid composition of the adipocere product which formed in the burial environments. The results suggest that adipocere can form under a variety of burial conditions. Several burial factors were identified as enhancing adipocere formation whilst others clearly inhibited its formation. This study acts as a preliminary investigation into the effect of the burial environment on the resultant preservation of decomposing tissue via adipocere formation.

Keywords: Adipocere; Fatty acids; Burial environment; Soil pH; Temperature; Moisture; Oxygen content

1. Introduction

The process of decay of human remains commences almost immediately following death and will usually pass through a number of stages including autolysis and putrefaction. Putrefaction can either proceed until the soft tissue has liquefied and the cadaver becomes skeletonised [1], or it can become arrested by later postmortem changes such as mummification or adipocere formation [2]. Mummification refers to the desiccation and preservation of soft tissue and usually occurs in hot, dry environments [3]. Adipocere formation refers to the process of conversion of body fat into a solid white substance [4].

Adipocere is a postmortem decomposition product which forms from the fatty tissue in a body. Adipose tissue comprises mainly triglycerides which during decomposition undergo hydrolysis to yield free fatty acids [4]. Hydrogenation of the free fatty acids will yield saturated fatty acids which, under the right conditions, form into adipocere [5]. Adipocere is comprised chiefly of the saturated fatty acids, myristic, palmitic and stearic acids. Unsaturated fatty acids [6,7], salts of fatty acids [8] and hydroxy- and oxo-fatty acids [6,9] have all been identified as constituents of adipocere.

Adipocere formation is of interest to forensic scientists as it has the ability to preserve the remains it encases by inhibiting decomposition. The degree of decomposition

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and differential preservation observed depends on the surrounding environment [10]. In a burial environment, a number of factors may inhibit bacterial growth, thus retarding decomposition and setting up favourable preservation conditions. The nature of the soil and sediments has been investigated [2,10] and its affect on adipocere formation reported previously by the authors [11]. Hence, whilst soil type is an important burial factor, it is not considered in this study.

The soil pH is important, as a highly acidic or highly alkaline environment will inhibit bacterial growth and thus, decomposition. The effect of pH on adipocere formation has been studied by Mant but a contradictory result was obtained. In Mant's laboratory experiments, the pH of the fluid in which the fat decomposed fell rapidly to produce an acidic environment in which adipocere formed [4]. However, his field studies identified adipocere formation on bodies in sealed coffins demonstrating a strong ammoniacal odour and presumably an alkaline environment [2].

The temperature of the burial environment can have a significant effect on decomposition as most bacteria thrive at an optimal temperature of approximately 37 °C [12,13]. Soil temperatures are typically much lower than this and bacterial growth may be inhibited. Putrefactive activity is greatly inhibited at temperatures below 10 °C or above 40 °C [12]. Such variations in temperature may be experienced in a shallow burial environment and will ultimately affect the extent of decomposition and preservation which occurs.

The oxygen content of the burial environment is likely to affect the rate of decomposition as the aerobic bacteria and fungi often found on decomposing remains require sufficient oxygen to survive and proliferate. An environment with minimal air circulation tends to delay decomposition and promote preservation [12,14]. Several studies have previously reported the requirement of an anaerobic environment for the successful formation of adipocere [5,15].

The formation of adipocere requires moisture for the hydrolytic conversion of triglycerides to free fatty acids. It was previously believed that adipocere formation could only occur in large bodies of water or water-logged environments. Studies have since shown that adipocere can form by utilising the moisture present within the body's tissues [2,4,10]. Hence, the presence of adipocere in a burial environment is not unusual even in a dry, soil environment.

The factors described represent some of the more common conditions present in a burial environment which may affect decomposition and preservation of human remains. Whilst the effect of these conditions on decomposition in soil has been studied in detail, their effect on adipocere formation in a burial environment is mostly based on observation. This study was conducted to experimentally investigate the burial factors of soil pH, temperature, moisture and oxygen content, with regard to their effect on adipocere formation. The results are useful in suggesting the possible effect of an individual burial factor on the rate and extent of the formation of adipocere.

2. Materials and methods

2.1. Adipocere formation

In order to determine the effect of particular burial conditions on adipocere formation, experiments were conducted in a laboratory environment so that the individual variables could be adequately controlled. The experiments were conducted in large decomposition containers which included a tap for draining purposes and an airlock seal for the release of gases present within the container. Due to ethical restrictions within Australia, the use of human adipose tissue was not a viable option for this study. Current considerations suggest that decomposing pigs are the next most reliable model [16,17] and this study therefore utilised domestic pigs which were reared for commercial use. Pig adipose tissue collected from the abdominal region and still containing muscle and skin was used for each experiment. To ensure adipocere formation occurred, each experiment included those factors known to enhance adipocere formation, namely sufficient adipose tissue, moisture, bacteria, and a relatively anaerobic environment. A control environment was created whereby the tissue was buried within a damp soil containing a loamy sand composition with pH approximately 5.2. Additional environments were prepared in the same manner with the individual variable of interest altered accordingly. The experiment was conducted in a temperate environment which averaged approximately 22 °C over a 12-month period.

The factors investigated in this study were pH (including the presence of lime), temperature, moisture and oxygen content. The pH of the burial environment was altered to investigate both acidic and alkaline environments. A highly acidic environment (pH approximately 2.4) was created through the addition of 10 M hydrochloric acid (HCl) to the soil. A mildly alkaline environment (pH approximately 8.5) was produced through the addition of 5 M sodium hydroxide (NaOH) to the soil whilst the highly alkaline environment (pH approximately 12.6) was created by covering the pig tissue in lime. The latter environment was included due to its occurrence in forensic situations whereby lime is often used to hide the odour and alter the rate of decomposition [18]. The pH values were determined using calibrated meters for measuring pH.

A variation in temperature was explored by investigating the extremes of cold and warm environments. A cold environment was achieved by placing the decomposition containers in a refrigerated environment held at 4 °C. A warm environment was created by placing the containers in a large water bath maintained at 40 °C. Both environments mimicked extremes in temperature variation when compared to the mild temperature of the control environment. The extremes aimed to represent temperature fluctuations which may be experienced in shallow grave sites of a forensic nature.

Moisture content was investigated by considering both a saturated and a dry soil environment. A dry environment was created by air drying and heating the soil to remove excess moisture before the fatty tissue was buried within. This procedure ensured that any moisture used in the formation of adipocere was extracted from the tissue and not from the surrounding soil. Conversely, a saturated environment was also investigated to determine whether excess moisture had any effect on the rate of formation of adipocere. Excess moisture was added to the soil before burial of the tissue and the moisture was monitored regularly to ensure a saturated environment was maintained for the entire decomposition interval. The moisture level was constantly monitored using a moisture meter with a scaled reading.

As an anaerobic environment is considered more conducive to adipocere formation, it was of interest in this study to determine the effect of an aerobic environment on adipocere formation in soils with a high redox potential. The effect of the aerobic burial environment was determined through comparison with the control burial environment which maintained an anaerobic environment for the duration of the study.

2.2. Sample collection

Three replicates of the control and other burial environments were prepared and the experiments conducted for a period of 12 months. Each container 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 were collected from each burial environment and placed in a sealed specimen container prior to analysis.

2.3. Infrared spectroscopy

Approximately 2 mg of adipocere was ground together with approximately 10 mg of powdered potassium bromide (KBr) using an agate mortar and pestle. The background spectra were run using approximately 20 mg of powdered KBr. The samples were investigated using a Nicolet Magna-IR 760 Fourier transform spectrometer equipped with a DTGS detector. Each sample was placed in a Spectra-Tech 3 mm microsampling cup for analysis. Reflectance spectra were obtained using a Nicolet diffuse reflectance infrared sampling accessory and the spectra were scanned over the frequency range 4000–500 cm^{-1}. For each sample, 64 scans were recorded with a resolution of 4 cm^{-1}.

2.4. Inductively coupled plasma–mass spectrometry

Five milligrams of adipocere sample was accurately weighed into a plastic screw-top tube and 300 μl each of concentrated HNO_3 (BDH Laboratory Supplies, UK), concentrated HCl (Riedel-de Haen, Germany) and H_2O_2 (BDH) added. The sample was heated until dissolved and allowed to cool. Upon cooling the 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 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. Gas chromatography–mass spectrometry

Two milligrams of adipocere sample was accurately weighed into a sterilised reactivial. One milliliter of chloroform (BDH Laboratory Supplies, UK) was added and the mixture sonicated for 15 min. The chloroform layer was drawn off, 250 μl 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 carrier gas was helium at a column pressure of 100 kPa. The initial column temperature was 100 °C and the initial time was 1 min. The temperature was then 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 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 acid. The unsaturated fatty acids, palmitoleic and oleic acid, 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 [19].

3. Results

3.1. Adipocere formation

At the completion of the experiment, adipocere was observed in the majority of burial environments, although the degree of formation varied. In a minority of cases, it could not be determined visually whether adipocere had formed and a chemical investigation by GC–MS was

required to confirm the presence or absence of saturated fatty acids related to adipocere. The control environment was successful in converting the entire mass of tissue into adipocere.

The samples collected from the acidic soil environment did not have an appearance consistent with adipocere. The skin was still evident and the decomposed remains demonstrated a pinkish-yellow colour with a crumbly texture. The mildly alkaline environment did, however, demonstrate a bright white adipocere product with an ammoniacal odour. Almost the entire mass of tissue appeared to have formed adipocere. The burial environment containing lime significantly inhibited decomposition and adipocere formation was not evident. The lime product had formed a shell encasing the tissue sample and a large quantity of the original tissue still remained. Adipose tissue was visible and appeared considerably fresh, even after a 12-month decomposition interval.

The warm burial environment generated a small mass of adipocere with a greyish-white appearance. The cold burial environment appeared to inhibit decomposition. The remains were visible and intact but demonstrated a pinkish colour and offensive odour. Adipocere could not be identified visually within the cold environment.

The saturated soil environment generated a large mass of adipocere with almost the entire tissue sample undergoing the conversion process. The adipocere samples collected were extremely wet and the burial environment produced an offensive odour. Most of the tissue sample had remained intact although some of the remnants were floating in the excess liquid. Similarly, a large mass of intact adipocere was also observed in the dry soil environment. The adipocere demonstrated a greyish-white colour but no offensive odour.

The aerobic environment was not conducive to adipocere formation as evidenced by the lack of adipocere, and more importantly, lack of tissue sample remaining after the 12-month decomposition interval. At the completion of the experiment it was observed that the entire sample had decomposed. Approximately 8 weeks after the experiments commenced, relatively small masses of fly larvae were observed on the surface of the soil within the aerobic burial environment. The presence of fly larvae was considered to mimic a natural decomposition environment and no attempt was made to control their presence.

3.2. Infrared spectroscopy

An infrared spectroscopic analysis was conducted for the samples collected from each burial environment to produce a preliminary lipid profile and identify the possible presence of adipocere. Peaks attributable to adipocere have been previously identified using infrared spectroscopy [20,21] and include triglycerides ($\approx$1740 cm^{-1}), fatty acids ($\approx$1700 cm^{-1}), and salts of fatty acids ($\approx$1576–1540 cm^{-1}). Fig. 1 illustrates the infrared spectrum characteristic of the adipocere collected from the control, mildly alkaline, warm, dry and saturated burial environments. These samples all demonstrated similar lipid profiles.

The control samples (Fig. 1a) contained a high concentration of saturated fatty acids as indicated by the band at 1700 cm^{-1}. A broad band representative of hydroxy fatty acids was observed in the region 2680–2670 cm^{-1} whilst small amounts of salts of fatty acids were present in the region 1576–1540 cm^{-1}. Strong C–H stretching bands were visible in the region 2950–2800 cm^{-1} and a small shoulder attributable to =C–H stretching of unsaturated fatty acids was observed to the left of this region. The lipid profile, as determined by the infrared spectrum, suggested that the conversion to adipocere had occurred in the control burial environment.

The additional spectra included in Fig. 1 demonstrate similar lipid profiles to the control samples with considerable abundances of saturated fatty acids and minor degrees of unsaturation. Hydroxy-fatty acids and salts of fatty acids appear to be present in all of the samples in varying amounts. Importantly, the spectra all lack evidence of triglycerides or large quantities of unsaturated fatty acids. Hence, the lipid profiles suggest that adipocere formation occurred in the mildly alkaline, warm, dry, and saturated burial environments.

Fig. 2 illustrates the infrared spectra for the samples collected from the cold and acidic burial environments which differed from the spectra shown in Fig. 1. The samples collected from the cold burial environment show a dominant band in their infrared spectrum near 1740 cm^{-1} which suggests an abundance of triglycerides. A smaller band at 1673 cm^{-1} provides evidence of unsaturated fatty acids and a weak band at 1539 cm^{-1} indicates small quantities of salts of fatty acids. The abundance of triglycerides and unsaturated fatty acids combined with the lack of saturated fatty acids in the lipid profile suggests that minimal decomposition of the adipose tissue occurred in the cold burial environment.

The spectrum for the samples collected from the acidic burial environment contains a band at 1700 cm^{-1} which is indicative of saturated fatty acids. A moderate band at 1634 cm^{-1} combined with a broad band in the region 3089 cm^{-1} also indicates the presence of unsaturated fatty acids. Bands relating to triglycerides and salts of fatty acids are lacking from the lipid profile. The lack of triglycerides demonstrates that the conversion process from adipose tissue to adipocere has occurred. However, the strong presence of unsaturated fatty acids suggests that the process did not occur to the same extent in the acidic burial environment when compared with the samples shown in Fig. 1.

The samples collected from the lime burial environment demonstrate a lipid profile which is not indicative of adipocere. The spectrum shown in Fig. 3 contains a band relating to triglycerides near 1745 cm^{-1} and a small band indicative of unsaturated fatty acids near 1652 cm^{-1}. The spectrum also contains small bands relating to salts of fatty acids in the region 1576–1540 cm^{-1}. The preliminary lipid

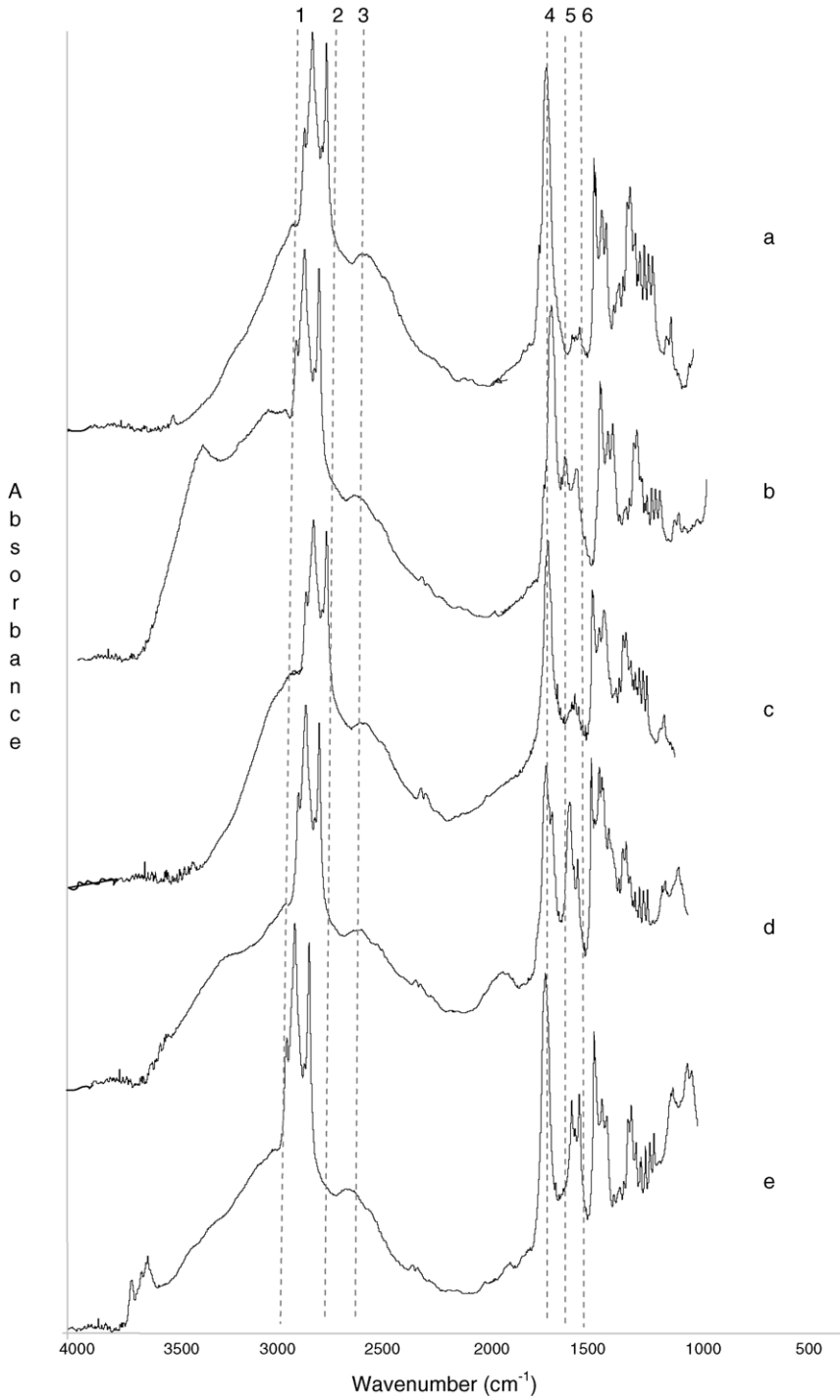

Fig. 1. Characteristic infrared spectra of adipocere samples collected from (a) control, (b) mildly alkaline, (c) warm, (d) dry, and (e) saturated burial environments. Dotted lines represent: (1, 2) C–H stretching (2950–2800 cm^{-1} region); (3) fatty acid O–H stretching (≈2670 cm^{-1}); (4) fatty acid C=O stretching (≈1700 cm^{-1}); (5, 6) fatty acid carboxylate C–O stretching (1576–1540 cm^{-1} region).

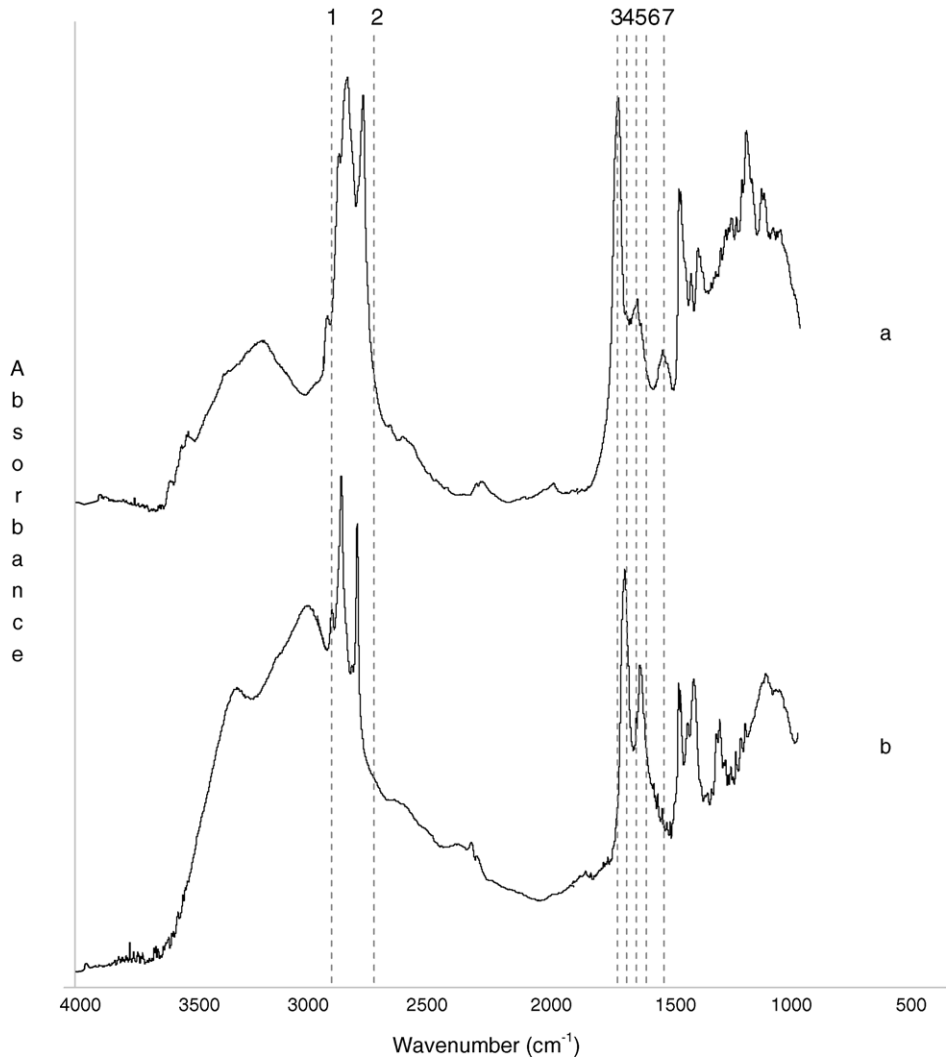

Fig. 2. Characteristic infrared spectra of samples collected from (a) cold and (b) acidic burial environments. Dotted lines represent: (1, 2) C–H stretching (2950–2800 cm^{-1} region); (3) triglyceride C=O stretching (≈1740 cm^{-1}); (4) fatty acid C=O stretching (≈1700 cm^{-1}); (5, 6) C=C stretching (1680–1600 cm^{-1} region); (7) fatty acid carboxylate C–O stretching (1576–1540 cm^{-1} region).

profile indicates limited decomposition, and no adipocere formation. However, the dominant band in the spectrum occurs near 1450 cm^{-1} and this band has not previously been identified in any of the samples. When compared with a reference spectrum of calcium hydroxide the same dominant band and additional correlating bands (e.g. lime O–H stretching band ≈ 2510 cm^{-1}) were identified. It was therefore determined that the collected samples were mostly lime with a limited amount of tissue attached. Due to their similarity in colour, sampling of the correct substance was difficult. The GC–MS analysis proved to be more useful in identifying the lipid composition of the samples collected from the lime burial environment as the interference from lime was not detectable.

3.3. ICP–MS

The infrared spectroscopic analysis identified salts of fatty acids in the majority of samples collected from the burial environments. In order to determine the types of salts of fatty acids present in the samples, they were further analysed using ICP–MS to identify the major cations. The salts which are generally considered to occur in adipocere are sodium, potassium, calcium, and magnesium salts of fatty acids [8,22]. In this study, the absolute abundance of each cation was calculated as a concentration in ppm for each adipocere sample. These values were converted to a relative abundance value (as a proportion of the total cations of interest) and an average calculated for each burial

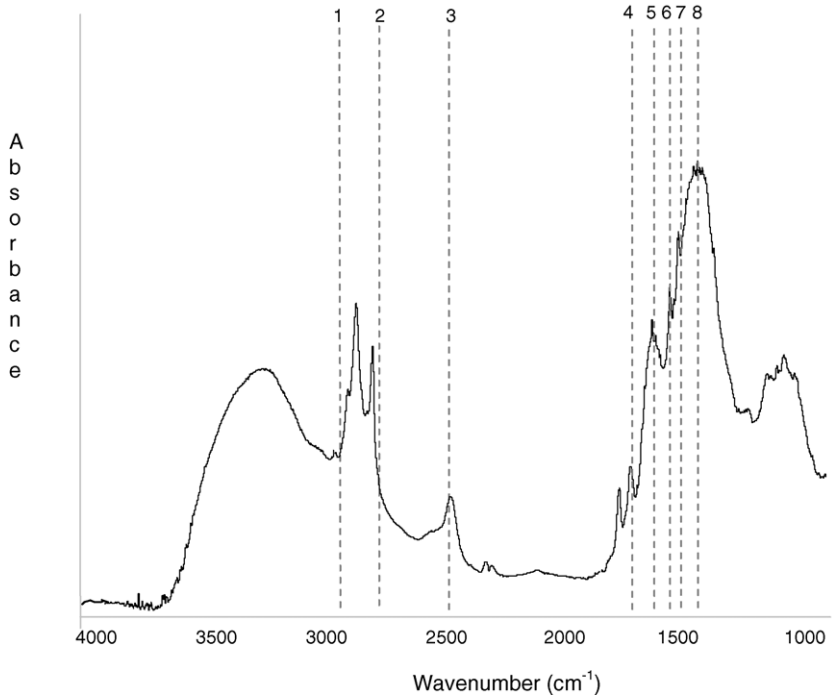

Fig. 3. Characteristic infrared spectrum of samples collected from the lime burial environment. Dotted lines represent: (1, 2) C–H stretching (2950–2800 cm^{-1} region); (3) lime O–H stretching (≈2510 cm^{-1}); (4) triglyceride C=O stretching (≈1740 cm^{-1}); (5) C=C stretching (1680–1600 cm^{-1} region); (6, 7) fatty acid carboxylate C–O stretching (1576–1540 cm^{-1} region); (8) lime C–O stretching (≈1450 cm^{-1}).

environment. Table 1 lists the average composition for the major cations identified in all of the samples.

The occurrence of salts of fatty acids in adipocere results from a cation exchange with the surrounding environment. As adipocere forms it will initially use sodium ions from the interstitial fluid, and later potassium ions from the cell or ambient water. Addition of sodium will produce a 'hard' soap with a crumbly texture while the addition of potassium results in the formation of a 'soft' soap with a paste-like texture [8]. In the later stages of adipocere formation, the sodium and potassium ions may be displaced by calcium or magnesium ions which are present in decomposition environments containing high mineral contents [8]. The soil burial environment may significantly interact with the decomposing tissue to promote or retard ion exchange.

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

Burial type	Na	K	Ca	Mg
Control	0.01	0.09	0.75	0.15
Alkaline	0.89	0.01	0.09	0.01
Acidic	0.01	0.06	0.63	0.30
Lime	0.00	0.00	0.99	0.01
Warm	0.03	0.16	0.42	0.39
Cold	0.09	0.25	0.27	0.39
Dry	0.01	0.04	0.64	0.31
Saturated	0.01	0.03	0.52	0.44

Relative abundance as a proportion of total cations determined.

The majority of samples demonstrated a high abundance of calcium ions followed by a moderate abundance of magnesium ions. In this study the formation of adipocere always occurred in a soil environment and cations were evidently able to exchange between the decomposing tissue and the surrounding soil. The sodium and potassium ions initially present in the tissue samples would have likely been replaced by calcium and magnesium from the surrounding soil environment. The extended decomposition interval of 12 months presumably allowed sufficient time for the exchange to take place. The majority of samples demonstrated the cations in the order Ca > Mg > K > Na which is in accordance with typical decomposition series seen in standard soils on 'micelles' (organic/inorganic colloids which attract the cations) [23]. Fig. 4 graphically demonstrates the relative abundance of the measured cations for those samples demonstrating the order Ca > Mg > K > Na.

A minority of samples demonstrated a cation profile with considerably reduced abundances of calcium ions when compared with the previously mentioned samples. The samples of adipocere collected from the mildly alkaline soil environment were unique in demonstrating an abundance of sodium ions. This abundance can be explained by the addition of concentrated NaOH to increase the alkalinity of the soil. An excess concentration of Na^+ ions would have

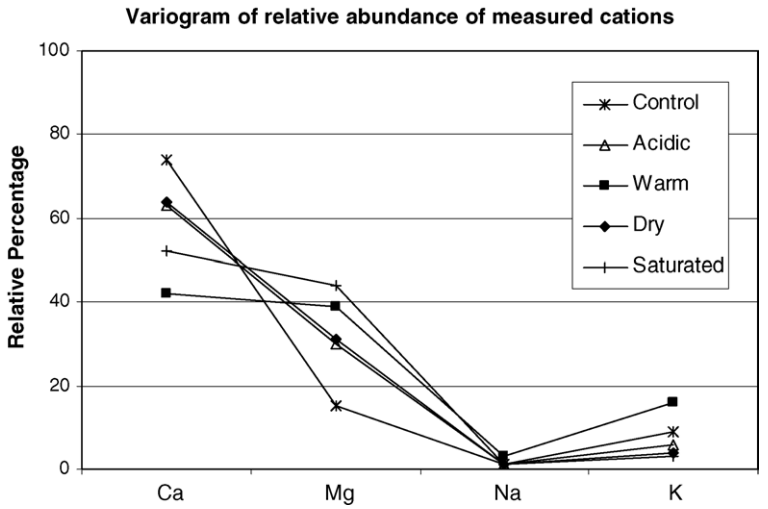

Fig. 4. Variogram of relative abundance of measured cations for samples showing similar cation compositions.

been present in the soil and available for exchange with those cations present in the tissue. Hence, an abundance of sodium salts of fatty acids was able to form in the adipocere and may account for the lack of calcium and magnesium ions observed.

The cold burial environment retarded decomposition in the tissue samples which would consequently slow the rate at which cations became available for exchange. The samples collected from this environment demonstrated a relatively equal abundance of potassium, calcium and magnesium ions. Cation exchange between the soil and tissue evidently occurred however the exchange process would have been severely inhibited by the cold environment. In this case, it was noted that adipocere formation was severely retarded.

The samples collected from the lime burial environment also demonstrated a unique cation profile. Although calcium was the abundant ion, almost no other ions were detectable within the samples. The abundance of calcium ions was most likely due to the addition of lime which consisted of calcium hydroxide and calcium carbonate. Consequently, an excess of Ca^{2+} would have been present in the surrounding environment and would have dominated the cation exchange process, thus explaining the lack of sodium, potassium and magnesium ions in the samples.

3.4. GC–MS

GC–MS was used to determine the fatty acid composition of the tissue samples. Table 2 lists the relative percentage composition of the fatty acids identified in the samples collected from the various burial environments. The fatty acid value represents an average of three repeat measurements for each of the replicate samples collected from the individual burial environments. Calculating the average of each set of samples was useful for giving an indication of the fatty acid composition for the decomposed remains. A characteristic fatty acid composition of the original adipose tissue is listed in Table 2 for comparative purposes.

The control samples demonstrated a composition which was high in saturated fatty acids and low in unsaturated fatty

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 ($C_{18:1}$)
Adipose	1.4	32.4	25.8	0.0	0.9	36.0
Control	5.1	58.7	29.2	1.1	0.0	6.0
Alkaline	3.3	60.8	29.1	0.0	0.0	6.9
Acidic	2.0	48.8	29.7	1.9	0.3	17.3
Lime	3.2	35.3	9.7	0.0	4.8	45.6
Warm	7.4	55.5	31.0	0.3	0.0	5.6
Cold	1.4	27.8	16.3	38.8	0.5	15.2
Dry	1.2	58.9	35.0	0.0	0.0	5.1
Saturated	4.2	65.2	27.5	0.7	0.0	2.5

acids. Palmitic acid represented the dominant saturated fatty acid followed by stearic acid. Small quantities of 10-hydroxy stearic acid were identified in the samples. Approximately 94% of the relative composition was comprised of saturated fatty acids. The samples collected from the control environment were not chemically stable due to the remaining oleic acid which could undergo further hydrogenation, but were representative of adipocere [19].

The percentage composition for the samples collected from the mildly alkaline burial environment demonstrated a profile very similar to the control samples. Slightly higher percentages of palmitic and oleic acid were observed, but overall the fatty acid composition was comparable. Further hydrogenation of oleic acid could have occurred to yield a stable chemical composition. The fatty acid composition was characteristic of adipocere and combined with the infrared spectroscopy results, confirms the formation of adipocere in the mildly alkaline burial environment.

The samples collected from the highly acidic burial environment demonstrate a retarded rate of formation when compared with the control samples. Whilst the relative percentages of myristic, stearic and 10-hydroxy stearic acid were comparable, a reduced percentage of palmitic acid was observed for the acidic samples. A small percentage of palmitoleic acid and an increased percentage of oleic acid were also evident. Consequently, the total relative percentage of saturated fatty acids present in the samples collected from the acidic burial environment was only 82% which is a notable decrease when compared with the control samples. The infrared spectroscopic analysis of the samples demonstrated an abundance of unsaturated fatty acids and suggested that the process of conversion from adipose tissue to adipocere had not occurred to the same extent as the control samples. The GC–MS results, when combined with the preliminary lipid profile, confirm that the conversion process was slowed, presumably by the highly acidic soil environment.

As demonstrated by the infrared spectroscopic analysis, the samples collected from the lime burial environment were not characteristic of adipocere. Interference from the lime meant that an accurate lipid profile could not be determined by infrared spectroscopy. However, the GC–MS results were able to demonstrate the effect of lime on the rate of decomposition. An extremely high percentage of oleic acid and a smaller percentage of palmitoleic acid remained in the samples. Although not included in Table 2, a small percentage of linoleic acid was also identified. Less than half of the total relative composition was comprised of saturated fatty acids, which was considerably reduced when compared with the control samples. The abundance of unsaturated fatty acids in the samples confirms that the conversion process to adipocere did not take place. The samples collected from the lime burial environment were more characteristic of adipose tissue which had undergone retarded decomposition.

The warm burial environment produced adipocere with a composition not dissimilar to the control samples. Slightly higher percentages of myristic and stearic acid and a slightly lower percentage of palmitic acid were observed, but the total relative composition of saturated fatty acids was identical. The GC–MS results, combined with the infrared spectroscopic lipid profile, confirm the formation of adipocere in the warm burial environment.

The cold burial environment, as previously suggested by the infrared spectroscopic profile, was not conducive to adipocere formation. A reduced percentage of palmitic and stearic acid was accompanied by a high percentage of oleic acid. Small amounts of palmitoleic and linoleic acid were also identified in the samples. However, the most notable difference in the fatty acid composition was the high abundance of 10-hydroxy stearic acid. Yan et al. [6] observed an increase in hydroxy stearic acid in the early stages of decomposition. The increase in 10-hydroxy stearic acid observed in this study may be due to the retarded rate of decomposition caused by the cold environment. The samples collected from the cold burial environment were not indicative of adipocere but more characteristic of adipose tissue which had undergone minimal decomposition.

The dry burial environment formed adipocere with a composition slightly varied when compared to the control samples. A moderately higher percentage of stearic acid and lower percentage of myristic acid was evident but the total saturated fatty acid composition was comparable. The saturated environment was also conducive to adipocere formation yielding an adipocere product with a high relative percentage of saturated fatty acids (approximately 98%). The samples collected from the saturated environment were almost chemically stable with only a minor proportion of unsaturated fatty acids remaining. The GC–MS and IR spectroscopic results confirm the formation of adipocere in both the dry and wet burial environments.

4. Discussion

In this study, the control burial environment involved the minimum factors necessary for the formation of adipocere (i.e. adipose tissue, moisture, bacteria and anaerobic conditions). The loamy sand environment was representative of a common soil and the formation of adipocere in this environment allowed it to be classed as the control for experimental purposes. All other burial environments included the same soil type and fatty tissue and were subsequently compared with the control environment to determine the effect of the particular burial factor on the extent of adipocere formation.

The mildly alkaline environment contained a higher pH than the control soil environment, but was still conducive to adipocere formation. The mildly alkaline pH of the soil would have been ideal for the survival and proliferation of destructive bacteria which would subsequently encourage decomposition of the adipose tissue. Once decomposition commenced, the moist, anaerobic conditions present in the

alkaline soil environment appeared to enhance adipocere formation. The control and mildly alkaline environments demonstrated adipocere formation within a pH range of approximately 5–9.

The highly acidic burial environment was not as conducive to adipocere formation, although adipocere did form in this environment. The presence of an extreme acidity appeared to retard the conversion process which yields adipocere. The retardation was likely due to the inability of many bacteria to live in a highly acidic environment. With a lower abundance of bacteria in the environment, the rate of decomposition and/or conversion to adipocere would have been slowed. The highly acidic environment was not entirely inhospitable to bacteria and the resultant product which formed was considered to have a fatty acid composition characteristic of adipocere.

The lime burial environment considerably inhibited decomposition and the formation of adipocere. The results obtained in this experiment correlate with results provided by Thew [18] which show that lime will retard the rate of decomposition if present in a burial environment. The most likely reason for the retardation was the ability of lime to prohibit the survival and proliferation of bacteria due to its extremely alkaline nature. A lack of bacteria in the surrounding environment would significantly affect the rate of decomposition and/or preservation of soft tissue as demonstrated in this study. Retardation of the decomposition process combined with the inhospitable bacterial environment would thus have reduced the likelihood of adipocere formation.

The warm burial environment maintained a temperature almost double the temperature in the control burial environment, but was shown to be just as conducive to adipocere formation. The variation in temperatures (22 and 40 °C) had no notable impact on the extent of conversion to adipocere. In this range of temperatures bacteria would not only survive, but also thrive in the tissue and surrounding soil. The thriving bacteria would have allowed for decomposition of the tissue to commence, and the favourable conditions surrounding the tissue would, thus, promote hydrolysis and hydrogenation. As there was sufficient moisture and an anaerobic environment, adipocere was able to form in the warmer temperatures. However, in extremely hot conditions (e.g. >40 °C) it is unlikely that adipocere would form due to the inability of bacteria to survive at higher temperatures and the rapid desiccation of the tissue.

The cold burial environment was not conducive to adipocere formation and inhibited decomposition to a certain degree. As discussed in Section 1, putrefactive activity is severely inhibited at temperatures below 10 °C and consequently decomposition was not extensive in the cold burial environment maintained at 4 °C. The preventative nature of the cold environment on bacterial activity and growth would account for the lack of adipocere identified in this environment. However, it must be noted that the burial environment investigated in this study was a cold soil environment and cannot be compared to cold icy environments such as those found in glaciers. Adipocere formation may occur in cold, glacial environments and is well documented in the case of the Iceman who was discovered in the Tyrolean Alps in 1991 [5,20].

The formation of adipocere in the control soil environment demonstrated that moderate moisture (but not in excess) was sufficient for adipocere formation to occur. However, the investigation of a dry soil environment demonstrated that no external moisture was necessary in the burial environment for the formation of adipocere. The results confirm prior suggestions that sufficient moisture is present in the decomposing tissue to yield adipocere [2,4,14]. Furthermore, the results suggest that the lack of moisture in a decomposition environment has no obvious effect on the formation of adipocere, allowing it to form at the same rate and to the same extent as adipocere which has formed in a moist (control) soil environment.

Whilst excess moisture was not necessary for the formation of adipocere, the presence of excess water in this study demonstrated an enhancement in the rate of conversion to adipocere. In a dry soil environment, the moisture would presumably be drawn from the tissue to aid the formation of adipocere until no moisture remained, resulting in desiccated tissue. However, in the presence of excess moisture, the process could theoretically continue without dehydration of the tissue and proceed until a chemically stable adipocere product formed. The negligible amount of unsaturated fatty acids, combined with the lack of triglycerides as demonstrated by the infrared spectroscopic analysis, confirms that near complete conversion to adipocere occurred in the saturated burial environment.

The anaerobic conditions present in the control burial environment were shown to be conducive to adipocere formation. Conversely, the aerobic burial environment was more favourable to decomposition and consequently did not form adipocere. The conversion process to adipocere was most likely prohibited by the rapid decomposition of the remains, and possible affects from the fly larvae observed in the first few weeks of the experiments. Furthermore, the presence of an aerobic environment favours oxidation of the fatty acids due to the action of bacteria, fungi and air [12,14]. Exposure to air would ultimately hasten the decomposition process and, thus, reduce the likelihood of preservation of the tissue.

5. Conclusion

This study demonstrates the effect of physical conditions on the rate and extent of adipocere formation from pig adipose tissue in a controlled soil, burial environment. The majority of factors considered were shown to be conducive to adipocere formation in a soil burial environment. The burial factors of cold temperature, lime and aerobic conditions showed a reduced or inhibited rate of adipocere formation when compared to the control environment,

whereas those of mildly alkaline pH, warm temperatures, and anaerobic conditions proved to promote the formation of adipocere. The results are useful for demonstrating the effect of an individual burial factor on adipocere formation but cannot be extrapolated to all burial environments. Further studies must be carried out to replicate these results and investigate the effect of combining the individual factors in a soil decomposition environment.

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