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TECHNICAL NOTE

Shari L. Forbes,[1] *Ph.D.; Barbara H. Stuart,*[2] *Ph.D.; Boyd B. Dent,*[3] *Ph.D.; and Sarah Fenwick-Mulcahy,*[2] *B.Sc.*

Characterization of Adipocere Formation in Animal Species

ABSTRACT: Adipocere is a soft white substance formed postmortem from fatty tissue in a decomposing body. In this preliminary study the formation of adipocere in soil was investigated for a number of animal species. Adipocere was formed from the fatty tissue of pig, cattle, sheep and rabbit. It was found that adipocere did not form from the fatty tissue of chicken or kangaroo in the time frame investigated. The issues being considered are relevant to the forensic examination of remains whose origin is otherwise uncertain or which are, in some way, related to human remains. Infrared spectroscopy and gas chromatography-mass spectrometry were used to characterise the composition of adipocere formed in the various species after different burial durations. Adipocere was observed to form at different rates among the species, but there was no distinct evidence of the fundamental composition varying between species.

KEYWORDS: forensic science, forensic taphonomy, adipocere, burial, non-human remains, infrared spectroscopy, gas chromatography-mass spectrometry

The formation of adipocere occurs by the alteration of the fatty tissue in decomposing remains into a solid white substance. Adipocere formation is characterised by the hydrolysis and hydrogenation of the neutral fats into a mixture of predominantly saturated fatty acids (mostly myristic, palmitic and stearic acids). In addition, unsaturated fatty acids (oleic and palmitoleic acid), salts of fatty acids, hydroxy and oxo fatty acids have all been identified as constituents of adipocere (1–7).

Several studies of pig adipocere formation have been reported (2,4), wherein it has been used as a model for human adipocere. In a burial environment, the surrounding conditions will effect the formation of adipocere (8) and include factors such as soil type, pH, temperature, moisture, and oxygen content (9,10). The formation of human adipocere has also been investigated (11–15). However, no studies of the nature of adipocere formation in other animal species have been reported. It is of interest to forensic scientists to know whether there is a species dependence in adipocere formation. An understanding of the process may provide valuable information regarding crime scene evidence, for example when adipocerous remains are otherwise unidentified as to species origin or when animal remains are part of forensic considerations. The purpose of the current study was a preliminary investigation of the nature and composition of adipocere formed in a number of common animal species, and comprised of two parts—an initial trial to generate adipocere (16), followed by its evaluation.

[1] Centre for Forensic Science, University of Western Australia.
[2] Department of Chemistry, Materials and Forensic Science, University of Technology, Sydney.
[3] Department of Environmental Sciences, University of Technology, Sydney.
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Adipocere was formed in soil by burial of fatty tissue samples of pig, cattle, rabbit, sheep, chicken and kangaroo, however, adipocere did not form on the latter two species. Infrared spectroscopy was used to provide a lipid profile of the adipocere samples, as well as for identifying the fatty acid salts. Gas chromatography-mass spectrometry (GC-MS) was employed as a method for the identification of fatty acids in the adipocere. These techniques were successfully used in earlier studies to characterise the extent of adipocere formation in soils (4,9,10,12).

Materials and Methods

Adipocere Formation

A section of fatty tissue containing some muscle and skin was utilised in the experiments and obtained from animals reared on domesticated diets specifically for commercial use. The species investigated were pig, cattle, sheep, rabbit, chicken and kangaroo. These species were chosen based on their distribution within Australia, availability, and possible likelihood of forming adipocere. Tissue was sourced from the abdominal region for the pig, cattle, sheep and rabbit samples, from the thigh region of the chicken, and the rump of the kangaroo. Adipocere was formed from the fatty tissue using a similar experimental method to that reported in earlier studies (9,10). 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. To ensure adipocere formation, each burial environment was prepared using those factors known to result in adipocere formation, namely sufficient adipose tissue, moisture, bacteria, and a relatively anaerobic environment (11,17). The environments were maintained in a temperate climate that averaged approximately 22°C over a 12-month period.

Sample Collection

Three replicate burial environments were prepared for each species being investigated. Adipocere formation was documented in situ at six, nine, and 12-month intervals. Samples of adipocere (where formation occurred) were collected at each burial interval and placed in separate sealed specimen containers for analysis.

Infrared Spectroscopy

Infrared spectroscopy was utilised to provide preliminary lipid profiles for those samples that appeared to form adipocere. All analyses were performed using the infrared spectroscopic method outlined in previous studies by the authors (4,9,10,12).

GC-MS Analysis of Adipose Tissue

GC-MS was utilized to determine the chemical composition of the original fatty tissue for the animal species being investigated. Approximately 2 mg of adipose tissue sample was accurately weighed into a sterilised reacti-vial. Approximately 1 mL of chloroform was added and the sample sonicated for 15 min. The chloroform layer was drawn off and evaporated under a flow of nitrogen. Approximately 1 mL of 0.1 M sodium hydroxide (NaOH) was added and the vial heated at 70°C for 5 min. After cooling, 2 mL of 0.2 M hydrochloric acid (HCl) and 1 mL of hexane was added and the mixture was extracted for 10 min. The upper hexane layer was drawn off and 250 μL of hexamethyldisilazane (HMDS) added. The vial was again heated at 70°C for 5 min and an aliquot removed for analysis by GC-MS.

GC-MS Analysis of Adipocere

GC-MS was also utilized to confirm the chemical composition of adipocere formation in the animal species being investigated. Approximately 2 mg of adipocere sample was accurately weighed into a sterilised reacti-vial. One ml 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. Approximately 250 μL of hexamethyldisilazane (HMDS) 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 using the GC-MS method outlined in previous studies (4,9,10,18). Table 1 reports the fatty acid concentrations for both adipose tissue and adipocere. For ease of interpretation, only those fatty acids identified in adipocere are included. Hence, the adipose tissue fatty acid composition does not report the polyunsaturated linoleic acid concentration that was present in most species and represents the remaining fraction of the total composition.

Results

Adipocere Collection

The burial environments were unsealed after 6, 9 and 12-month intervals. The pig, cattle and sheep samples demonstrated a soft white substance that appeared to be adipocere. Samples of the substance were collected from each container and placed in specimen containers for analysis. Due to experimental difficulties, rabbit samples were only collected at 6 and 12 months. Adipocere formation did not occur in the chicken or kangaroo samples.

Pig Sample Analysis

A sample of pig adipose tissue was collected prior to burial for the analysis of the triglycerides and the fatty acid content. Figure 1*a* illustrates the infrared spectrum of pig adipose tissue. Adipose tissue is known to comprise mainly triglycerides and the strong band at 1754 cm^{-1} is indicative of their presence in the sample (19). A weak shoulder on the lower wavenumber side of this band, combined with the band at 3005 cm^{-1}, indicate small amounts of unsaturated fatty acids.

The infrared spectrum of the pig sample collected after a 6-month burial duration is illustrated in Fig. 1*b*. The spectrum demonstrates a mixture of triglycerides (1737 cm^{-1}) and unsaturated fatty acids (1626 cm^{-1}). Strong bands relating to the salts of fatty acids are observed at 1575 cm^{-1} and 1539 cm^{-1}.

Figure 1*c* illustrates the infrared spectrum of pig tissue collected after a 9-month burial duration. A band indicative of triglycerides

TABLE 1—*Relative percentage (%) composition of fatty acids detected using GC-MS.*

Species & BD*		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 $C_{18:1}$
Pig	0	1	32	26	0	1	36
Pig	6	2 (0)	55 (6)	26 (2)	1 (1)	0 (0)	16 (2)
Pig	9	5 (2)	58 (2)	28 (4)	2 (2)	0 (0)	6 (3)
Pig	12	8 (7)	58 (7)	28 (4)	1 (1)	0 (0)	5 (3)
Cattle	0	6	36	31	0	3	22
Cattle	6	7 (2)	48 (8)	22 (3)	12 (6)	1 (0)	10 (2)
Cattle	9	3 (1)	60 (5)	36 (4)	0 (0)	0 (0)	1 (0)
Cattle	12	14 (6)	66 (11)	19 (3)	0 (0)	0 (0)	1 (1)
Sheep	0	3	25	30	0	4	39
Sheep	6	5 (1)	49 (7)	25 (6)	11 (6)	1 (0)	9 (3)
Sheep	9	2 (1)	55 (6)	38 (4)	2 (1)	0 (0)	3 (2)
Sheep	12	2 (1)	59 (6)	29 (3)	8 (3)	0 (0)	2 (1)
Rabbit	0	2	22	17	0	1	43
Rabbit	6	5 (1)	66 (4)	17 (2)	1 (1)	1 (0)	10 (3)
Rabbit	12	4 (1)	73 (2)	20 (1)	0 (0)	0 (0)	3 (2)
Human	A	12	49	28	0	2	10
Human	B	9	64	23	0	1	2

* Burial Duration reported in months.
(Standard deviation reported in brackets; $N = 3$. SD not included where sample size was insufficient).

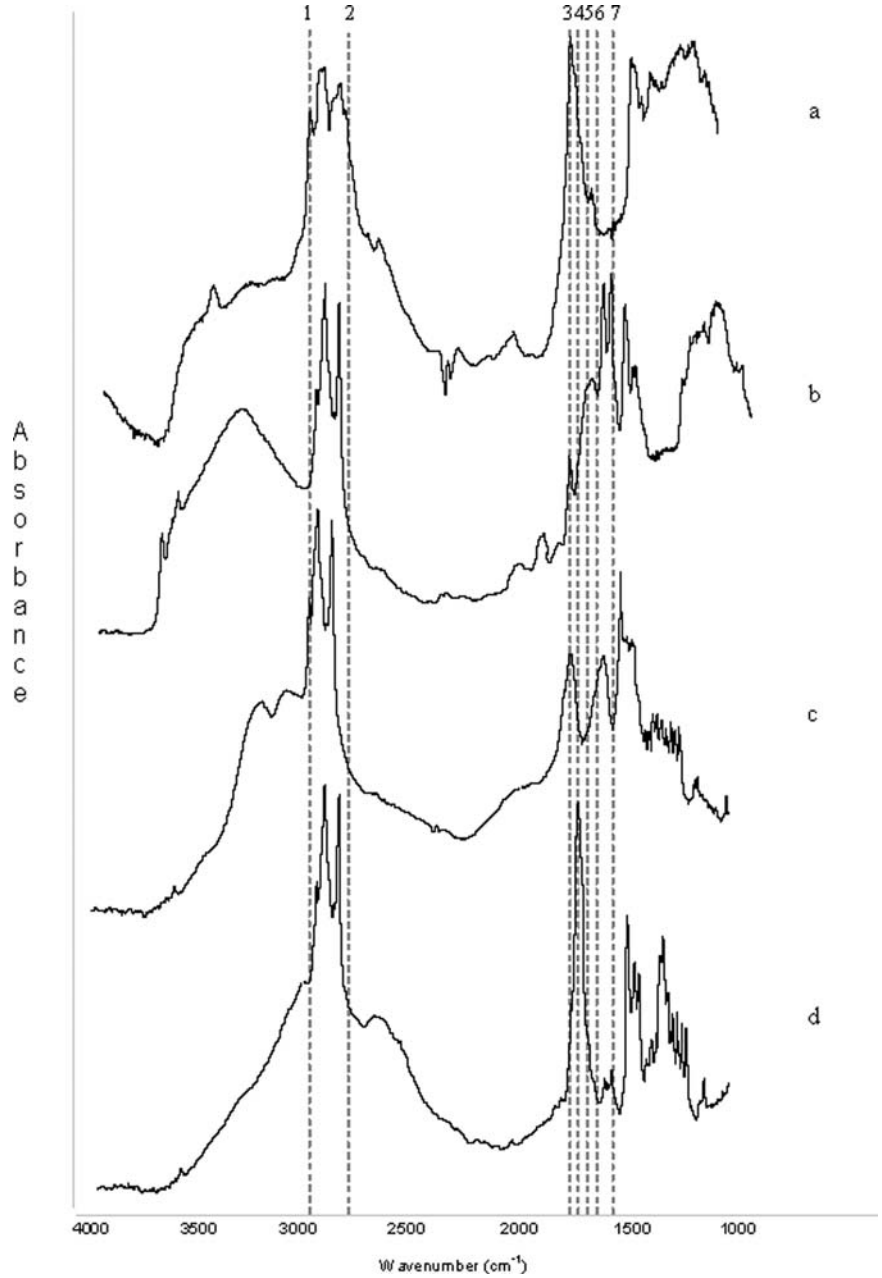

FIG. 1—*Characteristic infrared spectra of pig adipose tissue and adipocere collected after a) 0 months, b) 6 months, c) 9 months, d) 12 months burial duration. Dotted lines represent 1–2) C—H stretching (2950–2800 cm^{-1} region), 3) triglyceride C═O stretching (≈1740 cm^{-1}), 4) fatty acid C═O stretching (≈1700 cm^{-1}), 5) C═C stretching (1680–1600 cm^{-1} region), 6–7) fatty acid carboxylate C—O stretching (1576–1540 cm^{-1} region).*

is visible at 1709 cm^{-1}, but unsaturated fatty acid bands have completely disappeared. Only a broad band representative of salts of fatty acids can be seen at 1547 cm^{-1}.

After a 12-month burial duration, the pig adipocere sample appears considerably advanced in its development, as illustrated by the infrared spectrum in Fig. 1*d*. Strong saturated fatty acid bands are visible at 1701 cm^{-1} and a small band indicative of hydroxy fatty acids is evident in the region 2670–2660 cm^{-1}. The concentration of salts of fatty acids is significantly reduced with only a small band visible at 1540 cm^{-1}.

The pig samples were also analysed by GC-MS immediately following their collection. Table 1 lists the average percentage composition of fatty acids detected in the pig samples at various burial durations. An obvious increase in the concentration of palmitic acid was evident over the 12-month period. A slight increase in the concentration of stearic acid occurred while the concentrations of myristic and 10-hydroxystearic acid varied. Additionally, a distinct reduction in the concentration of oleic acid is coupled with the complete loss of palmitoleic acid. By the completion of the experiment, adipocere formation in pig tissue was evident by the characteristic fatty acid ratio (4,18).

Cattle Sample Analysis

A sample of cattle adipose tissue was collected prior to its burial and analysed by infrared spectroscopy (Fig. 2*a*). The main band at 1744 cm^{-1} is attributable to the high concentration of triglycerides

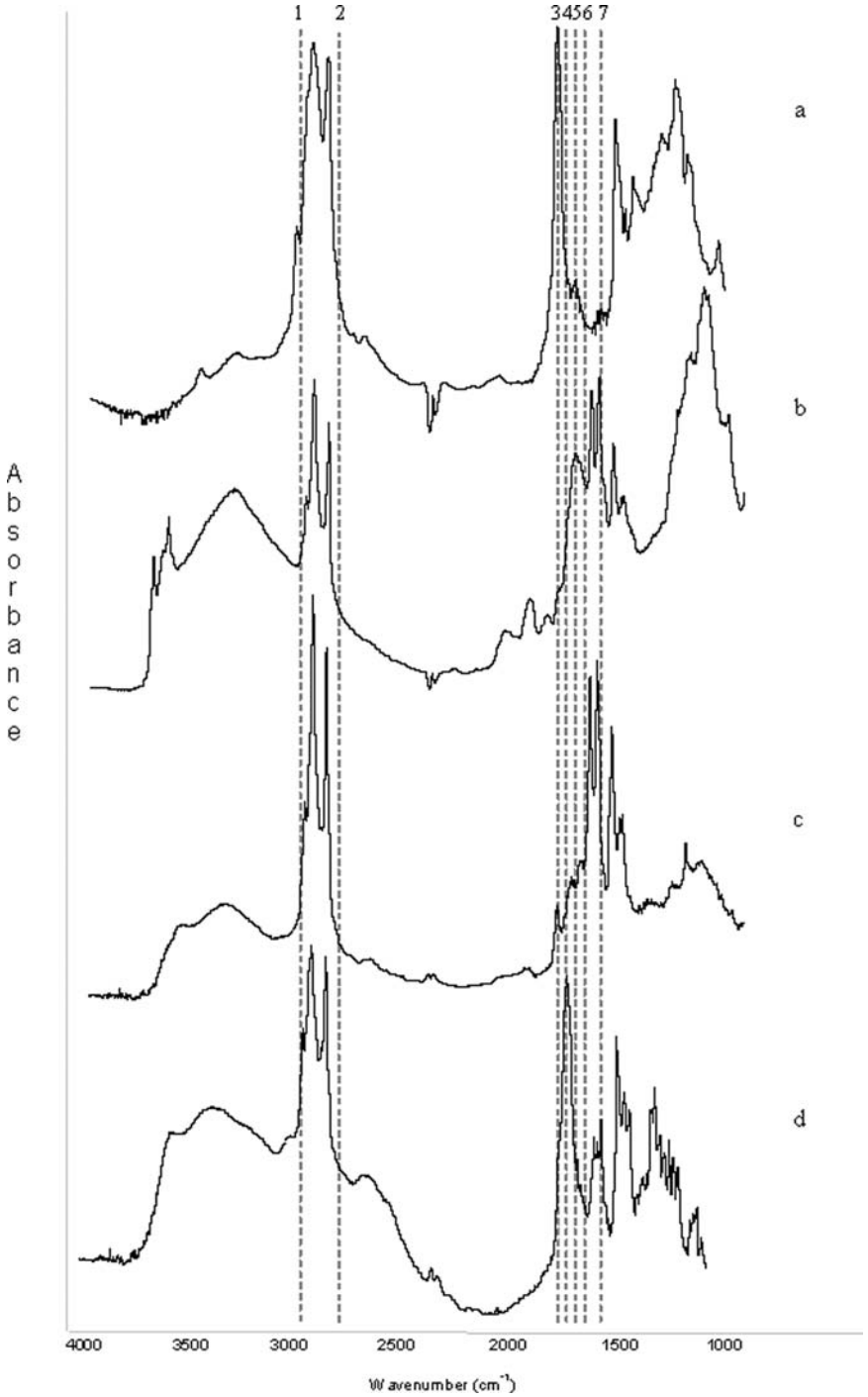

FIG. 2—*Characteristic infrared spectra of cattle adipose tissue and adipocere collected after a) 0 months, b) 6 months, c) 9 months, d) 12 months burial duration. Dotted lines represent 1–2) C—H stretching (2950–2800 cm^{-1} region), 3) triglyceride C═O stretching (≈1740 cm^{-1}), 4) fatty acid C═O stretching (≈1700 cm^{-1}), 5) C═C stretching (1680–1600 cm^{-1} region), 6–7) fatty acid carboxylate C—O stretching (1576–1540 cm^{-1} region).*

present in adipose tissue. Small amounts of unsaturated fatty acids are evident as bands at 3004 cm^{-1} and 1657 cm^{-1}.

The sample of cattle tissue collected after 6 months burial duration produced the infrared spectrum illustrated in Fig. 2*b*. The spectrum illustrates an unsaturated fatty acid band at 1656 cm^{-1} and a small shoulder at the higher wavenumber end of this band that is indicative of triglycerides. Bands due to the salts of fatty acids are observed at 1576 cm^{-1} and 1539 cm^{-1}.

Figure 2*c* illustrates the infrared spectrum of the cattle sample collected after a 9-month burial duration. Strong bands relating to salts of fatty acids can be seen in the region 1576–1540 cm^{-1}. Small bands relating to triglycerides and unsaturated fatty acids are present, but the intensity of the bands is considerably reduced.

Figure 2*d* illustrates the infrared spectrum of adipocere collected after 12 months. The spectrum shows strong C-H stretching bands (2950–2800 cm^{-1}) and a strong band relating to saturated fatty acids (1701 cm^{-1}). Bands at 1576 and 1540 cm^{-1} are evidence of salts of fatty acids. A lack of bands attributable to triglycerides or unsaturated fatty acids indicates the extent of conversion to adipocere.

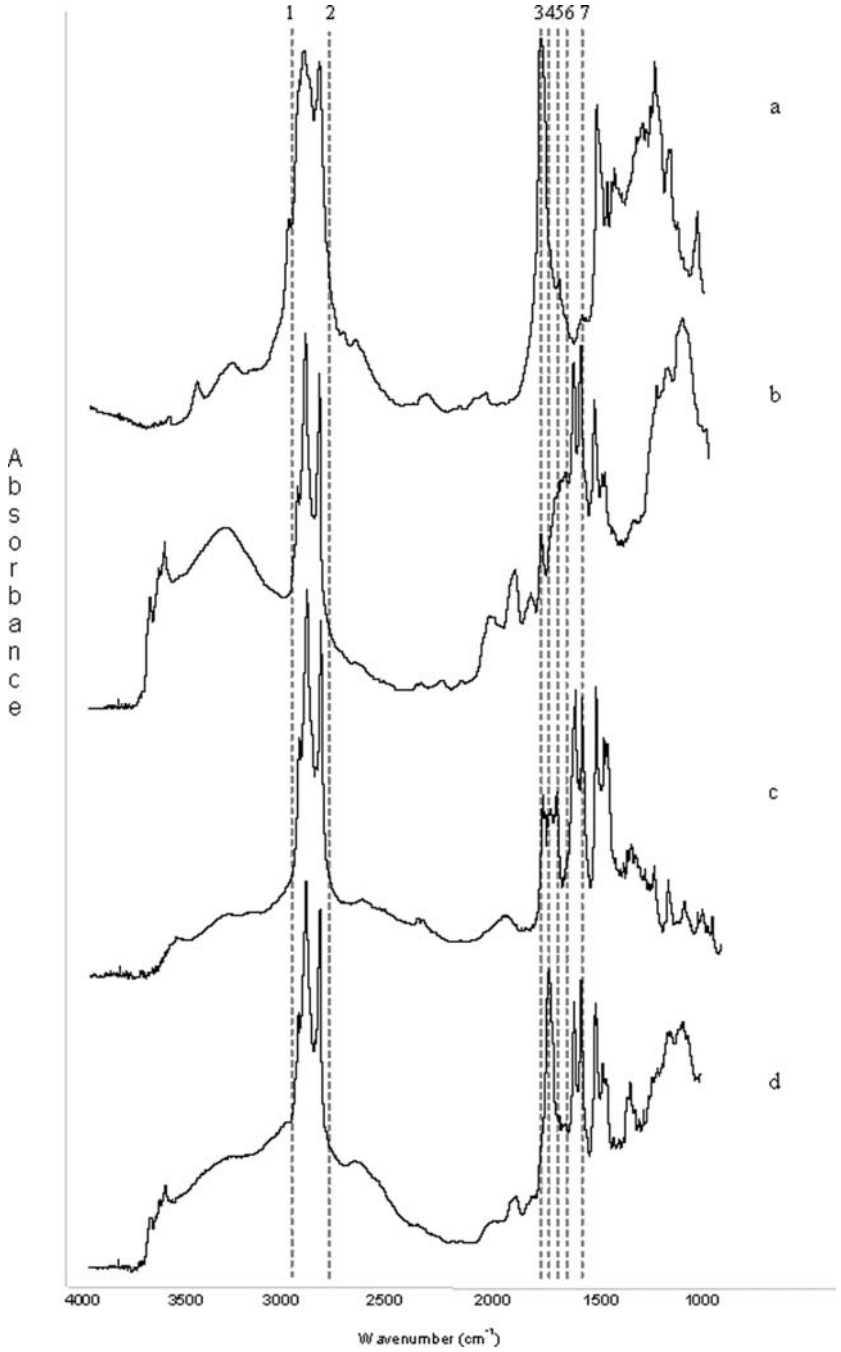

FIG. 3—*Characteristic infrared spectra of sheep adipose tissue and adipocere collected after a) 0 months, b) 6 months, c) 9 months, d) 12 months burial duration. Dotted lines represent 1–2) C—H stretching (2950–2800 cm^{-1} region), 3) triglyceride C═O stretching (≈1740 cm^{-1}), 4) fatty acid C═O stretching (≈1700 cm^{-1}), 5) C═C stretching (1680–1600 cm^{-1} region), 6–7) fatty acid carboxylate C—O stretching (1576–1540 cm^{-1} region).*

Table 1 lists the average percentage composition of fatty acids detected by GC-MS in the cattle samples at various burial durations. An increase in the concentration of palmitic acid with a concomitant decrease in the concentration of oleic acid is observed presumably due to β-oxidation and hydration of the double bond (1). A reduction in the concentration of palmitoleic acid has also occurred. The concentrations of myristic and stearic acid varied and 10-hydroxystearic acid was rarely identified.

Sheep Sample Analysis

A sample of sheep adipose tissue was analysed using infrared spectroscopy prior to burial and produced the infrared spectrum illustrated in Fig. 3*a*. The most intense band is observed at 1743 cm^{-1}, due to the triglycerides that comprise the majority of adipose tissue.

The sheep sample collected after the 6-month burial duration produced the infrared spectrum shown in Fig. 3*b*. The sample still contains triglycerides as evidenced by the small band at 1738 cm^{-1}. However, bands relating to fatty acids and salts of fatty acids are also present. A small shoulder at 1632 cm^{-1} is indicative of unsaturated fatty acids. At 9-months' burial duration, the infrared spectrum in Fig. 3*c* is similar to Fig. 3*b*. The spectrum still contains small bands relating to triglycerides and unsaturated fatty acids, however, a small band indicative of saturated fatty acids is also observed at 1700 cm^{-1}. The spectrum for the 12-month sample (Fig. 3*d*)

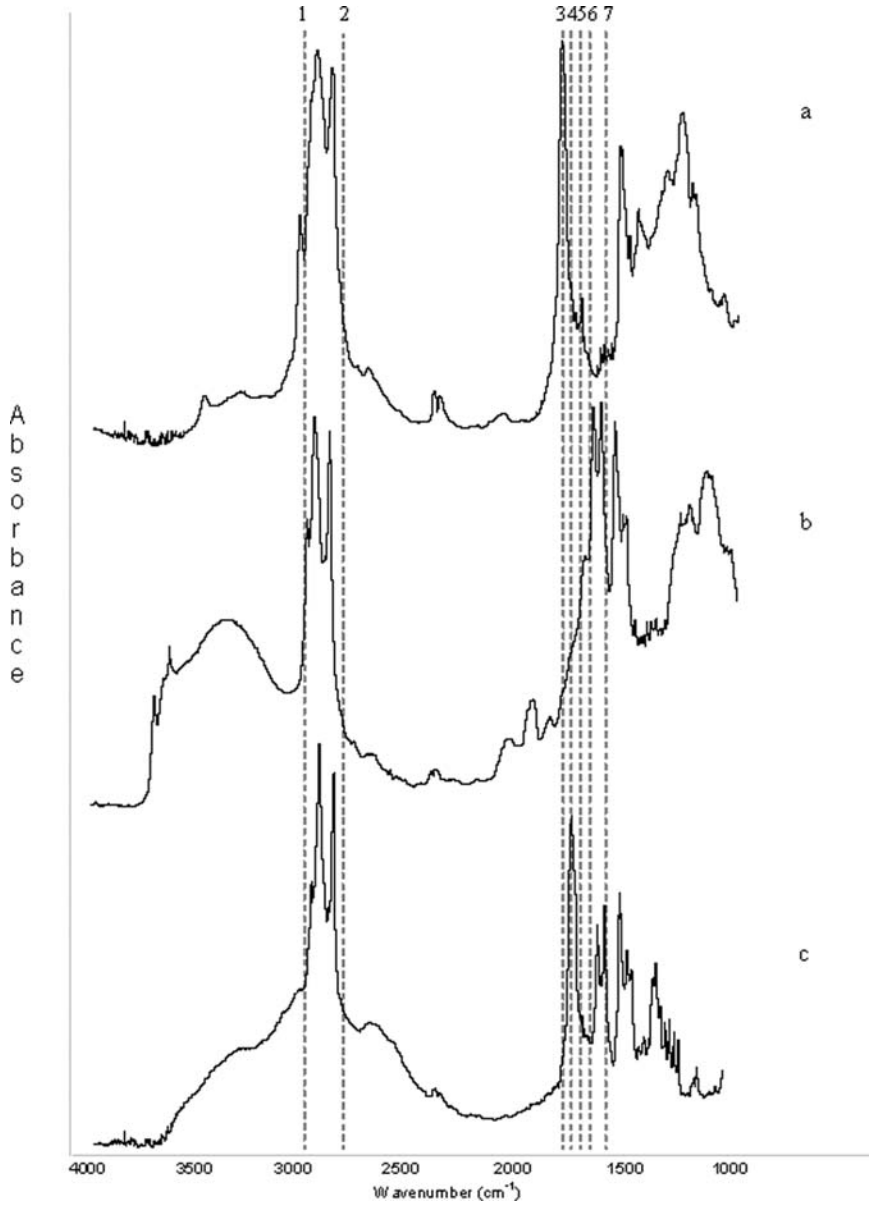

FIG. 4—*Characteristic infrared spectra of rabbit adipose tissue and adipocere collected after a) 0 months, b) 6 months, c) 12 months burial duration. Dotted lines represent 1–2) C—H stretching (2950–2800 cm^{-1} region), 3) triglyceride C═O stretching (≈1740 cm^{-1}), 4) fatty acid C═O stretching (≈1700 cm^{-1}), 5) C═C stretching (1680–1600 cm^{-1} region), 6–7) fatty acid carboxylate C—O stretching (1576–1540 cm^{-1} region).*

provides evidence that adipocere formation has occurred in the sheep tissue. Strong bands due to saturated fatty acids (1700 cm^{-1}) are evident, along with bands attributable to salts of fatty acids (1576 and 1540 cm^{-1}).

Table 1 details the relative percentage composition of the fatty acids detected in sheep tissue using GC-MS. As with the previous species investigated, an increase in the concentration of palmitic acid is observed with an attendant reduction in the concentration of oleic acid. The concentration of palmitoleic acid decreased with time until it was no longer present whilst the concentrations of myristic, stearic and 10-hydroxystearic acid varied over time. The ratio of fatty acids present at the completion of the experiment is indicative of typical adipocere formation.

Rabbit Sample Analysis

The infrared spectroscopy analysis of rabbit adipose tissue produced the spectrum shown in Fig. 4*a*. The spectrum is typical of adipose tissue in that it contains a strong band relating to triglycerides at 1747 cm^{-1}. A small band attributable to unsaturated fatty acids is observed at 1653 cm^{-1}. The infrared spectrum for the 6-month rabbit samples (Fig. 4*b*) shows a distinct lack of triglycerides however unsaturated fatty acids are evidenced by a small shoulder at 1650 cm^{-1}. The bands relating to C-H stretching (2950–2800 cm^{-1}) and salts of fatty acids (1576–1540 cm^{-1}) dominate the spectrum. The sample of adipocere collected after 12 months burial duration demonstrates an advanced composition, illustrated by the spectrum in Fig. 4*c*. A strong band indicative of saturated fatty acids is seen at 1700 cm^{-1}. This band is stronger than the nearby bands relating to salts of fatty acids.

Table 1 lists the relative fatty acid concentration of the rabbit samples detected using GC-MS over varying burial durations. A rapid increase in the concentration of palmitic acid is again evidenced with a simultaneous rapid decrease in the concentration of oleic acid. Conversely, the concentrations of myristic, stearic and

10-hydroxystearic acids remain relatively constant. Palmitoleic acid disappears with time.

Discussion

Of the six species investigated, adipocere formed on four, namely pig, cattle, sheep and rabbit in the contexts investigated. Both chicken and kangaroo were determined to be unfavourable for adipocere formation. It is hypothesised that the lack of adipocere formation in theses species is due to a lack of sufficient fatty tissue. Notably, kangaroo meat is regularly marketed as being a lean-meat alternative both in Australia and globally.

The infrared spectroscopy results were useful in demonstrating the conversion process from adipose tissue to adipocere. The early presence of triglycerides was progressively replaced by unsaturated fatty acids. The resultant presence of saturated fatty acids and salts of fatty acids was evidence of adipocere formation in the various animal species.

Comparison of the fatty acid content in the neutral fats of the original tissue samples demonstrated significant differences between species. In particular, the palmitic, stearic, and oleic acid concentrations varied considerably. Studies by Kagawa et al. (19) show comparable differences between species and provide species identification using fatty acid analysis.

A comparison of the samples collected after 6 months' burial duration show variations between species type with rabbit demonstrating the highest concentration of palmitic acid. This finding is predictable as, although rabbit originally contained the lowest palmitic acid concentration, it contained the highest oleic acid concentration in the original fatty tissue, which could be subsequently converted to palmitic acid during the hydrolysis and hydrogenation process. The pig and rabbit samples were dissimilar to each other and the other species investigated, however the sheep and cattle samples showed similar fatty acid compositions.

A comparison of the fatty acid composition for the samples collected after the 9-month burial duration showed distinction. As this set of samples lacked a representative rabbit sample, only pig, cattle and sheep samples were compared. Variations were again evidenced in the fatty acid composition of each species however these variations were not consistent with those observed in the 6-month set of samples. Increases in the concentration of palmitic and stearic acid, combined with a decrease in the oleic acid concentrations, was evidence that adipocere formation was occurring in all species. However, consistent variations were not observed for each species between the 6 and 9-month burial durations.

A comparison of the species' samples collected after the 12-month burial duration provided similar results. High concentrations of palmitic acid coupled with low concentrations of oleic acid demonstrated that the adipocere product was becoming progressively more chemically stable. This set of samples demonstrated the most noticeable variations and had the sample set been analysed independently, it may have appeared that adipocere formation was species specific. However, in conjunction with the 6 and 9-month burial duration comparisons, the variations amongst fatty acid composition of different species are inconsistent.

A recent study by the British Geological Survey analysed adipocere collected from a burial pit containing pig and cattle carcasses following the 1967 foot and mouth epidemic (20). The study identified the same fatty acids (with the exception of lauric acid) as used in this study to characterise adipocere. The fatty acid compositions of the adipocere samples from the burial pit were comparable to the compositions identified in this study. However, the adipocere samples were not able to be distinguished as being from pig or cattle or both, and the difficulty in discriminating between adipocere from animal species is further demonstrated.

Furthermore, the authors have conducted chemical investigations of adipocere collected from human gravesites over a range of burial durations (12). Although the burial durations were considerably longer for the human burials than the animal burials, comparable fatty acid compositions were observed in some instances (see Table 1). Consequently, the inability to chemically distinguish between human and animal species could result in an incorrect determination of species origin in adipocere samples where this factor was unknown. The research outlined in this study was, in fact, based on a forensic investigation involving the discovery of adipocere samples wrapped in plastic bags in an outdoor environment (Forbes: personal communication). Determination of species type was in dispute as to whether the samples were of human or deer origin. A chemical analysis of the unknown samples produced a fatty acid composition that was almost identical to adipocere samples known to be collected from a pig in a previous study. The location of the remains made it unlikely that pig was the species in question and as a result, the species origin of the samples could not be conclusively determined by a chemical investigation.

Although this preliminary study was not able to detect significant specific differences between the fatty acid compositions of various animals, it may still be possible to demonstrate that adipocere is species specific following further studies. This pilot study utilised the GC-MS method that identifies only specific fatty acids known to comprise adipocere from earlier studies. A variation of the GC-MS method that investigates more components present in an adipocere sample may provide information regarding an additional fatty acid derivative not detected in this study. Studies by Takatori et al. (5) have identified derivatives such as oxo- and keto-fatty acids, whilst a study by Adachi et al. (21) found epicoprostinol in adipocere. It is possible that one or more of these derivatives are present in the adipocere of different species and that their concentrations vary consistently.

Conclusions

Of the six species investigated, adipocere did not form on two of the species, chicken and kangaroo. These species were determined to be unfavourable to adipocere formation due largely to the minimal amount of fatty tissue present in the original tissue samples. Studies have previously noted the requirement of sufficient adipose tissue to be present for adipocere to form (11,17). This study found that pig, cattle, sheep and rabbit fatty tissue are able to form adipocere in soil. The adipocere that formed in different species was similar in appearance and chemical composition. Evidence was provided that the formation of adipocere occurs at different rates for each species. However, there is no evidence that the fundamental composition is species-dependent. Comparison with other studies of pig, cattle, deer, and human adipocere samples, confirmed the difficulty in distinguishing between adipocere samples of different animal species. Further research in this area may be able to identify alternative fatty acids or derivatives of fatty acids that are common to one species and not another. Studies involving the extraction of DNA from adipocere to determine species origin are proceeding.

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Additional information and reprint requests:
Shari Forbes, Ph.D.
Centre for Forensic Science (M420)
University of Western Australia
35 Stirling Highway, Crawley 6009
Australia