Forensic Science International 195 (2010) 168.e1–168.e6

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

Forensic Anthropology Population Data

Comparison of adipocere formation in four soil types of the Porto (Portugal) district

Nuno Durães [a], Débora Cortez [a,c], Manuel Algarra [a], Francisco G. Sánchez [b], José E. Rodríguez-Borges [c], Iulius Bobos [a], Joaquim C.G. Esteves da Silva [c,*]

[a] Centro de Geologia do Porto, Faculdade de Ciências da Universidade do Porto, R. Campo Alegre 687, 4169-007 Porto, Portugal
[b] Departamento de Química Analítica, Facultad de Ciencias, Universidad de Málaga, 29071 Málaga, Spain
[c] Centro de Investigação em Química [CIQ(UP)], Departamento de Química, Faculdade de Ciências da Universidade do Porto, R. Campo Alegre 687, 4169-007 Porto, Portugal

ARTICLE INFO

Article history:
Received 11 May 2009
Received in revised form 22 September 2009
Accepted 15 November 2009
Available online 14 December 2009

Keywords:
Adipocere
Free fatty acids
Water content
Soil types
Forensic Anthropopulation Data

ABSTRACT

Four typical soils of the Porto (Portugal) area were characterized and used to study the decomposition of buried pieces of pork meat under controlled laboratory experiments (an 8 month experiment with a relatively high soil moisture and a 1 month experiment with relatively low soil moisture). The soils types were: organic, sandy, gravel and clay-gravel soils. Soils were characterized for their grain size distribution, pH, water content, organic matter percentage and mineral composition. Four free fatty acids (myristic, palmitic, oleic and stearic) were analysed (using a methodology based on an extraction step followed by a derivatization reaction and high performance liquid chromatography analysis) in soil samples as a sign of adipocere formation. The direct sensorial analysis of the buried sample residues and the free fatty acids profiles of the sampled soils showed that sandy and clay-gravel soils (in a low moisture environment) slowed the normal decomposition process promoting the formation of adipocere. Nevertheless, this apparent soil effect is indirect and a consequence of the different water retention and permeability of the soils. Thus, the water content of the soils is a crucial factor for adipocere formation.

1. Introduction

The Directorate of the municipal cemeteries of the Porto city reports the existence of sections where corpses do not decompose after the regular resting time, some of them for more than 50 years, which causes management problems, due to the impossibility of grave reuse and deactivation of these sections as burial grounds. The visual inspection of the unskeletalized corpses suggests that the cause for these problems is related to an inhibition of post-mortem changes due to adipocere formation [1–3].

In one of the cemeteries of Porto, the Agramonte cemetery, a possible cause for the problematic sections is the existence of an aquifer that passes underneath the cemetery. Indeed, the most problematic section contains an active well, and has already been deactivated as a burial site. This observation agrees with previous reported results that show that the presence of water is one of the most important factors for adipocere formation [4,5]. However, in other cemeteries without aquifers the apparent inhibition of post-mortem changes due to adipocere formation is also detected in some sections and, in these cases, the soil type characteristics must be the critical factor. Previous literature reports showed that the soil characteristics affect the process of adipocere formation [4,6]. However, other factors such as burial environment, soil pH, temperature, moisture and oxygen content have been investigated [7,8].

The parent material of the soil was considered the most important characteristic to assess its suitability as a graveyard [1–3]. The following characteristics have been positively correlated with adipocere formation or degradation problems: loam-enriched moraine material; poorly permeable materials such as schist; sediments from the weathering of red sandstones; "black-coloured" soils; soils with poor drainage (clay and loamy enriched soils); and soils with a high proportion of fine particles (<6.3 μm) (clay enriched soils).

Adipocere is a post-mortem anaerobic decomposition product that is composed mainly of saturated fatty acids myristic, palmitic and stearic acids [8–12]. Other unsaturated fatty acids (for example, oleic acid), salts of fatty acids (mainly calcium salts) and hydroxyl fatty acids (for example, 10-hydroxy stearic acid) have been identified as constituents of adipocere [11,12].

This paper presents the results of a study about the effect of four soil types characteristic of the Porto district, namely organic (OS), sandy (SS), gravel (GS) and clay-gravel (CGS) soils, on the decomposition of pork meat under controlled experimental

* Corresponding author. Tel.: +351 220402569; fax: +351 220402659.
E-mail address: jcsilva@fc.up.pt (Joaquim C.G. Esteves da Silva).

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doi:10.1016/j.forsciint.2009.11.010

conditions. Pork meat must be used in these types of studies due to ethical criteria. Two experiments, under semi-anaerobic conditions, were made using different soil moisture conditions (wet and dry) by burying pork meat in a soil sample placed inside an air tight plastic container for 1 month in the dark. Other wet experiments were done that took 4 and 8 months. The soil samples were characterized and four free fatty acids were monitored as an indication of adipocere formation in the soil samples: myristic, palmitic, oleic and stearic acids.

2. Materials and methods

2.1. Reagents

Free fatty acids standards (myristic, palmitic, stearic and oleic acid) were supplied by Sigma–Aldrich Química SA (Spain). The derivatizing chiral agent, (*R*)-(+)-1-phenylethylamine, dichloromethane, methanol (HPLC grade) and ethylic ether were purchased from Merck (Darmstadt, Germany) and Panreac (Barcelona, Spain), respectively. All compounds were analytical-reagent grade and the purities were stated to be higher than 99%. Stock standard solutions in methanol were used for HPLC measurements.

2.2. Synthesis of the corresponding standard fatty acid amide derivatives (FAA)

Myristic, palmitic, oleic and stearic ceramides (FAA) were prepared from the corresponding fatty carboxylic acids (FFA). These acids were converted into the corresponding carboxylic–carbonic anhydride intermediate by treatment with ethyl chloroformate (2 equiv.) and triethylamine (2 equiv.) under argon using dried dichloromethane as a solvent (10 mL/4 mmol of fatty acid) following the methodology described in the literature [13]. The corresponding anhydride intermediate, which was not isolated, was concentrated by vacuum and was reacted with (*R*)-(+)-1-phenylethylamine (1.2 equiv.) under argon using dried dichloromethane as a solvent (20 mL/4 mmol of initial fatty acid), giving the corresponding fatty amide. The resulting organic mixture solution was washed with 1 M HCl (4× 10 mL), the organic layer was washed with brine (20 mL) and dried with anhydrous sodium sulphate. Removal of the solvent using a rotary evaporator yielded the corresponding pure ceramide quantitatively (Scheme 1). The structure of the pure ceramides was checked by ^{1}H NMR-400 MHz (CD_3Cl) and molecular mass analysis. Standard FAA solutions were prepared by dissolving 10 mg of synthesized derivatized FAA in 10 mL methanol, stored at 4 °C and in absence of light. Working solutions were prepared by dilution with methanol.

2.3. Soil samples

Four soil types representative of the Porto district were collected. These soils have a similar genesis from granite and/or metamorphic rocks. Their macroscopic characterization easily discriminated the four types of soils (Table 1) as organic (OS); sandy soil (SS); gravel (GS); and clay-gravel (CGS). The discriminating properties are mainly related to weathering minerals (clays) and organic matter content, which will affect the pH and permeability (water and air circulation) of the soils.

2.4. Soil characterization methods

Raw soil samples were analysed for their grain size distribution using a standard sieve series in a mechanical shaker. Mineralogy was carried out by X-ray diffraction (XRD) using a Rigaku Miniflex D/max. – C series automated diffraction system equipped with a Cu Kα radiation. Samples were analysed in the range 4–70° 2θ, using a 1° divergence slit, a step increment of 0.05° 2θ and a counting time of 10 s/step.

The pH of all soil samples were measured by mixing soil with deionised water (1:5 of V_{soil}:V_{water}) after 10 min stirring followed by a resting period of 2 h. pH was measured using a HI255 HANNA millivoltmeter, equipped with a HI1131B HANNA combined glass electrode, previously calibrated with two buffer solutions (4.01 and 7.01) from HANNA. The percentage of organic matter was determined by rigorous weighing by incinerating 2 g of dry soil with the following temperature program: 105 °C for 48 h at 375 °C for 1 h; 600 °C for 6 h. The moisture percentage was determined by weighing the soil before and after a 24-h period at 105 °C.

Table 1
Soil sample macroscopic characterization.

Soil classification	Macroscopic characterization
Organic (OS)	Dark coloured soil with high percentage of organic matter.
Sandy (SS)	Brownish soil with a high percentage of sand collected near maritime coast.
Gravel (GS)	Greyish-white soil with a relatively high weathering of primary minerals.
Clay-gravel (CGS)	Reddish soil with a relatively high weathering of primary minerals.

2.5. Pork decomposition experiments

Two sets of experiments were performed that correspond to: (i) relatively high moisture in the soil (simulating winter weather conditions); and (ii) relatively low moisture in the soil (simulating low rainy seasons). The first experiment (relatively high moisture) was performed during an 8-month period and the second experiment (relatively low moisture) during a 1-month period. The experiment with relatively high moisture was performed since the average precipitation in the Porto area is about 100 mm, or higher, in the fall, winter and spring months. The four soils were used in the experiments and triplicate samples were always assembled.

The first experiment consisted of the burial of small pieces (a parallelepiped of about 1 cm high) of pork loin with no visually detected fat (10.9 ± 0.5 g), followed by the addition of deionised water (about 50 mL), in a plastic hermetic box (15 cm × 15 cm × 5 cm) – the box was closed and kept inside a closed black plastic bag protected from light for the pre-selected time of 1, 4 and 8 months period. The volume of the soil occupied about 80% of the volume of the box and the piece of meat was placed in the centre of the box with about 1.5 cm of soil above it. Consequently, at the beginning of the experiments, the environment inside the containers contain some oxygen – these conditions somewhat simulate burial under about 1 m soil of a dead body inside a coffin, which is a normal practice in Portugal. Also, in Portugal, it is not normal practice to treat the corpses prior to burial (e.g. embalming). After a period of time after burial, which is highly dependent of the environmental conditions, the unsealed wood coffin degrades and soil mixes with the decomposing body affecting the subsequent decomposition pathways.

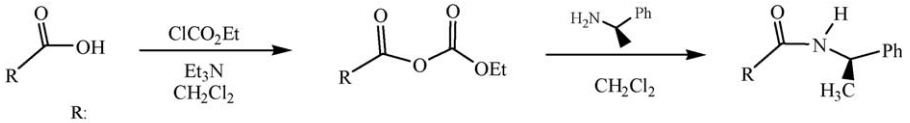
The second experiment followed a similar design as the first but no water was added. In addition to pork loin with no visible fat, the second set used pork fat and skin – in this case only a 1-month long experiment was performed.

The pork samples were obtained from a local butcher and were from the same animal. Consequently any observed differences in the decomposition results of the pork samples were due to factors other than the samples alone.

After the pre-selected resting time the boxes (three boxes for each soil) were opened and a visual inspection was performed. Also, a randomly selected portion of the soil inside the plastic box was removed for the soil analysis. The rest of the sample present inside the box was stored inside a −20 °C freezer until free fatty acid analysis could be performed.

2.6. Free fatty acids analysis

Soxhlet extractions were performed using variable amounts of analytically weighed soils, without previous treatment. Samples (30–75 g) were extracted under reflux with 1:1 dichloromethane–ether solution (100 mL) for 3 h. The samples were cooled to room temperature, mixed with anhydrous sodium sulphate and filtered. The extracts were dried using a rotary evaporator at 40 °C and reduced pressure. The extracts were transferred into a Schlenk tube reactor, equipped with a magnetic stirrer and maintained in an inert atmosphere (argon) during the reaction after the addition of anhydrous dichloromethane (2 mL/50 mg of isolated sample), ethyl chloroformate (40 μL/50 mg of sample) and triethylamine (50 μL/50 mg of sample), for 3 h at room temperature. After that, a gentle vacuum was applied, to evaporate all solvents and excess reactants. (*R*)-(+)-1-phenylethylamine was added



Scheme 1. Reaction scheme of the transformation of free fatty acids into ceramides.