Lipid Analysis on Potential Grave Soil Products

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ABSTRACT: Determination of time of death for a buried body is difficult. Based on a number of studies, it has been recognised that the lipid distribution extracted from soil grave may provide significant information to determine the time of death. For this study, a control laboratory burial experiment under tropical weather was conducted. The pig fatty flesh was allowed to decompose for a month and the soils were sampled at five different sampling points. The extracted lipids were analysed to determine their concentration. The modification of Bligh-Dyer extraction method was used. These soil lipids were classified into three groups, which were saturated fatty acid (SAFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA).

KEYWORDS: Lipids; Fatty acid; Potential grave product; Post-mortem interval

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INTRODUCTION

These days, people used entomology to establish the postmortem interval (PMI) (Gennaard, 2012). The distribution, biology and behavior of insects found where a body has been discovered are the main components of forensic entomology, providing information on when, where and how a crime was committed or a person died (Catts & Goff, 1992; Anderson & Cervenka, 2002). Principally, the calculation of egg laid time in the life cycle of insects has been used to consider the minimum time of death (Anderson, 1996; Singh *et al.*, 2014). Many factors affect the rate of decomposition and the variation in temperatures has demonstrated a remarkable impact on the amount of fatty tissues converted to adipocere (Polson *et al*, 1985; Forbes *et al.*, 2005b). At 37 °C the optimum temperature bacteria have thrived in the tissue and surrounding soil. The suitable surrounding conditions of the tissue may promote the hydrolysis and hydrogenation the human tissue (Bereuter *et al.*, 1997; Takatori, 1996; Yan, 2001).

Generally, the decomposition process of human remains will pass through a number of stages. For this study, the decomposition has been described as taking place in five stages, i.e. initial (0-3 day), putrefaction (4-10 day), black putrefaction (10-20 day), butyric fermentation (20-50 day), and dry decay (50-365 day) (Vass, 2001). Human tissues need two and three months to decompose completely in warm ambient and longer in cold environment (Yan *et al.*, 2001). However, other environmental factors may cause the completion of the decomposition be longer. Throughout decomposition process, some organisms in soil depleted the body tissues and provided a condition suitable for the putrefaction stage to occur. These organisms migrated from the soil into the human flesh in the later stages of decomposition (Evans, 1963).

Adipocere can preserve the body, including their facial features and injuries (Kumar *et al.*, 2009). Recent study found that adipocere may also help to determine the PMI, time has elapsed since a person has died (Gennaard, 2012). A clandestine grave can also be located using adipocere since the yellowish waxy material from the body leaks into the underneath soil (Gunn, 2009). Adipocere may also reveal the condition of surrounding environment. The texture of the

adipocere formed on the cadaver can give some insights into the rate of decomposition (Vass, 2001). The legitimate factors can be useful in forensic investigation and estimation of PMI (O'Brien *et al*., 2007 ; Ubelaker & Zarenko, 2011). During decomposition process, fatty tissues can form adipocere on fatty body. Adipocere contains the mixture of predominant saturated and unsaturated fatty acids as constituents then leach out into the soil (Takatori, 1996).

To date, lipid analysis was used to determine the concentration ratio of fatty acid contents per sampling point to estimate the PMI. A control burial laboratory experiment was conducted using soil of rubber plantation to establish preliminary results of potential grave products. A modified Bligh and Dyer extraction method was used to extract the soil lipids. This method utilises a solvent combining chloroform and methanol in acidic conditions. The presence of acid improved the extraction of free fatty acids (Cavonius, 2014). This method showed a higher recovery of total lipid from the tissues (Bligh & Dyer, 1959).

METHODOLOGY

Simulated Burial Experiment

The experiment was designed to stimulate burial in a shallow grave. 28 ml volume vials were used to mimics the shallow grave. It was fully filled-up with soil and 30 g pig fatty flesh was buried and allowed to decompose according to the specific burial intervals. The vials were exposed to ambient environment and the moisture content had been checked consistently. The soils were collected accordingly for each of the sampling point: Day zero (D0), Day first (D1), Day three (D3), Day fifteen (D15) and Day thirty (D30).

Soils with adipose tissue were stored in a freezer at -20 °C prior analysis. Then soils were freeze-dried and transferred into airtight sample glass bottle and stored.

Preparation of Buffered Water and Bligh-Dyer

300 ml of double distilled water was transferred into a separating funnel and 2.04 g potassium dihydrogen phosphate (KH_2PO_4) was added to create a 0.05 M solution. The pH was adjusted to pH 7.2 by addition of sodium hydroxide (NaOH) pellets and the mixture was extracted with 3 x 50 ml dichloromethane (DCM). The Bligh-Dyer solvent mixture was made up using buffered water: chloroform: methanol with ratio 4:5:10, respectively.

Modified Bligh-Dyer Extraction

Approximate 0.5 g soil was transferred into a Pyrex culture tube and the dry weight obtained. 3 ml of DCM/methanol (2:1 v/v) was added into the soil, then spiked with 100 µl internal standard nanodecanoic and sonicated for 15 min, followed by centrifuging for 5 min (~3000 rpm). The supernatant was transferred into a clean vial. The process was repeated three times for 2 ml of DCM/ methanol (2:1 v/v). The soil then treated with 3 ml of Bligh-Dyer solvent, sonicated for 15 min and centrifuged (~3000 rpm, 5 min). The supernatant was transferred into the same vial. The extraction was repeated with 3 x 2 ml Bligh-Dyer solvent. To break the organic phase, 2 ml each of buffered water and chloroform were added to the supernatant and the mixture centrifuged for 1 min (~3000 rmp). The organic layer then transferred into a clean vial. This process was repeated with 3 x 2 ml chloroform. The solvent was evaporated from the resulting TLE solution under a gentle flow of nitrogen, and the resultant TLE stored in a freezer below -20 °C.

Instrument Analysis

Samples were analysed using a gas chromatography- flame ion detector (GC-FID) (GC-2010 plus, Shimadzu). Separation was performed with BPX70 polar capillary column (30 m x 0.25 mm internal diameter, 0.25 µm film thickness). Helium was used as a carrier gas. After injection at 50 °C, the oven temperature was raised to 100 °C at a rate 10 °C min^{-1} and held for 5 min., then to 250 °C at 4°C min^{-1}, and finally held constant for 20 min. The flame ionization was held at 255°C. Peaks were identified by comparing their retention times with those of authentic standards (Supelco Inc.).

RESULT AND DISCUSSION

Total lipid extract (TLE)

Figure 1 showed the mass of total lipids extracted from the soils. The mass of extractable lipid components were found to range between 0.0752mg/g and 0.1674 mg/g soil dry weight. The mass TLE increased from D0 to D3 and decreased afterward, with slightly increased at D30. D3 soil, corresponding to initial stage, contained the highest mass of TLE i.e. 0.1674 mg per soil dry weight. This trend clearly showed a rapid decomposition occurred between D0 and D3. Moreover, the decreasing in mass of TLE after D3 indicated a slowdown in the decomposition process. These days correspond to black putrefaction and butyric fermentation stages (Vass, 2001).

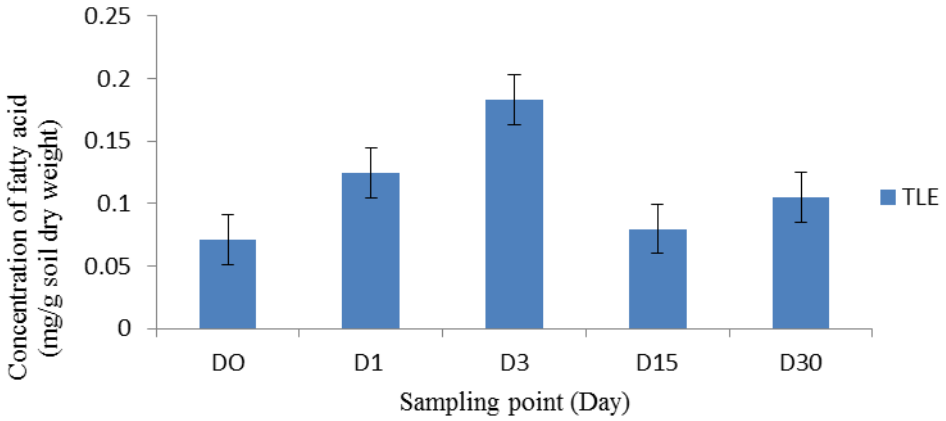

Figure 1: Mass of total lipid extract (TLE) for each sampling point

Fatty acid analysis

Table 1 showed the concentration of fatty acid obtained from associated soils. The fatty acids were classified as saturated fatty acid (SAFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA).

The higher concentrations of SAFAs were observed for the extracts of D0 and D30, corresponding to initial and butyric fermentation stages, respectively. Furthermore, the total concentration of palmitic acid ($C_{16:0}$) was higher than stearic acid ($C_{18:0}$) between D0 and D3, corresponding to initial stage of decomposition process. The concentration of palmitic acid found to range between 0.24 mg/g and 0.23mg/g soil dry weight. Whilst, the concentration of stearic acid decreased from 0.28 mg/g to 0.11 mg/g per soil dry weight, similar trend as has been observed from previous study (Forbes *et al.*, 2002). However, the total concentrations for these saturated

fatty acids were lower compared to the study conducted by Forbes (2005) where the fatty acids were extracted from the adipocere and not the soil.

Table 1: Concentration of fatty acids in soil (mg/g soil dry weight)

SAFA	**D0**	**D1**	**D3**	**D15**	**D30**
$C_{16:0}$	0.24±0.21	0.23±0.11	0.23±0.30	0.39±0.43	0.65±0.42
$C_{17:0}$	0.23±0.30	0.02±0.30	0.23±0.20	0.45±0.21	0.47±0.30
$C_{18:0}$	0.28±0.01	0.06±0.20	0.11±0.11	0.08±0.03	0.43±0.51
$C_{23:0}$	0.53±0.42	0.02±0.12	0.04±0.02	0.09±0.03	0.18±0.21
$C_{24:0}$	0.62±0.30	0.03±0.22	0.06±0.02	0.19±0.20	0.17±0.12
Total	**1.90±0.34**	**0.36±0.10**	**0.67±0.21**	**1.20±0.35**	**1.90±0.42**
MUFA	**D0**	**D1**	**D3**	**D15**	**D30**
$C_{16:1}$	0.14±0.62	0.01±0.01	0.01±0.01	0.01±0.02	0.01±0.01
$C_{18:1n9}$ (c)	-	0.32±0.45	0.19±0.11	0.32±0.30	0.39±0.41
$C_{20:1n9}$	0.61±0.71	0.16±0.55	0.11±0.34	0.16±0.21	0.25±0.32
$C_{22:1n9}$	-	-	0.03±0.12	-	-
$C_{24:1}$	0.68±0.80	0.11±0.21	0.15±0.11	0.10±0.12	-
Total	**1.43±0.65**	**0.59±0.34**	**0.48±0.23**	**0.58±0.25**	**0.64±0.35**
PUFA	**D0**	**D1**	**D3**	**D15**	**D30**
$C_{18:3n6}$	0.53±0.08	0.02±0.23	0.10±0.32	0.14±0.23	0.15±0.41
$C_{18:3n3}$	0.22±0.62	0.44±0.32	0.08±0.03	0.09±0.04	0.11±0.02
C20:2	0.59±0.02	0.06±0.07	0.15±0.03	0.16±0.21	0.42±0.35
C20:3n3	0.59±0.03	0.02±0.23	0.07±0.03	0.14±0.34	0.23±0.02
C22:2	-	0.02±0.24	0.09±0.02	0.14±0.03	0.25±0.21
Total	**2.34±0.31**	**0.56±0.22**	**0.49±0.12**	**0.76±0.32**	**1.16±0.43**

The concentration of palmitoleic acid, i.e. one of the constituent in adiopcere, was high at DO (0.14 mg/g soil dry weight). Whilst, the concentrations of this acid were unchanged and considerably low between D1 and D30 (0.01 mg/g soil dry weight). The demonstrated trend was agreed with the findings from previous studies where the formation of unsaturated fatty acid in sandy and silty soils was higher at initial stage and then inhibited until butyric fermentation stage (Forbes *et al*., 2005).

The total concentrations of PUFA were higher at D0, corresponding to initial stage. The concentration of PUFA decreased between D1 and D15, corresponding to putrefaction and black putrefaction stages. The concentration of these PUFA then increased at D30 (butyric fermentation stage). The observation may indicate a rapid decomposition at the early stage of decomposition. Comparatively, the concentration of PUFA found to be higher compared to SAFAs and MUFAs. The formation of PUFA at initial stage was rapid and lower at putrefaction stage until butyric fermentation stages. Whilst, in D0, the absent of docosadienoic acid ($C_{22:2}$) compound was observable. This observation may due to unstable chemical composition sources of the soil (Forbes, 2005).

CONCLUSION

Lipids composition has contributed as indicator in soil to determine the PMI. The present study suggests the concentration of lipids content during decomposition process might vary among the interval of death time and decomposition stages. The saturations of fatty acid show the significant values of lipids concentration to be used as parameter for post mortem interval. However, the extended period of laboratory experimental will continue to meet the timescale of decomposition process of fatty flesh completed.

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