The Study of Natural Saponification Processes in Preservation of Human Corpses

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The natural course of evolution of the human body after death includes autolysis, putrefaction and skeletonization. Under specific environmental conditions, the body may bypass this natural path, leading to preservation, such as saponification (adipocere), lignification, mummification or refrigeration, comprising the entire body or being limited only to some parts of it. All these preservation processes have a chemical substrate, and the identification of their components may be useful in many forensic circumstances, such as clandestine activity and identification of victims in mass disasters, when pentanoic, butanoic, hexanoic acid, butanoic acid-butyl ester, hexanoic acid-ethyl ester, indole, dimethyl disulphide may be used to train human remains detection dogs. The authors present the case of a 73 years old woman who was found dead in a sewage collection basin 4 months after her disappearance in July. The autopsy revealed a mixture of cadaveric processes, some parts being disintegrated, with putrefaction and skeletonization, while other parts were preserved by saponification: areas of adipocere were found on the neck, thorax and the anterior part of the abdomen. In some instances the saponification of the corpses makes identification possible and preserves violence marks, but the combination with putrefaction burdens these goals. The identification in this case was possible using the teeth formula, keeping in mind that tooth and bones are the most resistant to putrefaction. Even though saponification makes difficult the estimation of postmortem interval, investigations of its chemical composition may be useful in this direction, as the epicoprostanol-cholesterol ratio proved to increase with the increasing of postmortem interval.

Keywords: saponification, death, environment, chemical processes, preservation

The usual way of evolution for the human body after its vital functions have stopped and death is installing is towards total dissolution, starting with autolysis and going to putrefaction, which continues until the body is reduced to a skeleton [1, 2]. This process may last different periods of time, up to hundreds of years [3].

Yet, sometimes the environmental conditions where the corpse is found or the particularities of the body create a deviation from this usual course, and the corpse conserves in an unusual manner. The natural ways of for conserving the bodies that have a chemical substrate are: saponification (adipocere), mummification, lignification and refrigeration.

Adipocere appears as a result of saponification of the fat tissue. Its name varies: grave wax [2, 3], corpse wax, fat of graveyards [4], *cera cadaverica* [5].

Although some authors define adipocere as a process of mummification [4], *or fatty wax type of mummification* [6], in a strict sense, these processes are different both by the environmental conditions necessary for their occurence, and also by the corpse aspect. However, it is not excluded to notice the association of the decomposing processes with the conservation ones in the same corpse [1].

The interest in studying cadaveric saponification appeared over two centuries ago, when Fourcroy and Thouret noticed that certain bodies buried in a graveyard in Paris, exhumed when it was closed, did not decompose, but were preserved [3]. Fourcroy was also the one who gave the name to this process, by joining these two words from Latin - *adeps* meaning fat and *cere*, meaning wax [1].

As a macroscopic aspect, in most cases saponification occurs on limited areas of the body, but there are also cases when the whole body is affected [1]. Basically any organ with fat tissue, regardless of its size, may undergo saponification [7].

In the initial stages adipocere is soft, with a fatty aspect, but as time passes, it becomes hard, crumbly and resembling limestone [1, 4]. Although its colour is off-white, the products resulting from hemolysis can confer colours ranging from white to pink and grey or greyish-green. The smell of adipocere, recorded by Evans in the year 1962, is *earthy, cheesy and ammoniacal* [1].

Initially saponification occurs in the skin (after about 6 weeks), it later expands to the muscles (after about 3-4 months), and the extremities will be turned into adipocere after a few months up to a few years [3]. The soft tissues in the child bodies contain more fat tissue and this favours the appearing of adipocere [8].

The factors that favour adipocere to form depend on the environment where the body is kept and also on the particularities of the body.

The environmental factors are represented by:

-mineral composition and soil aeration [3];

-increased humidity- soils that retain water (like clay soils) favour adipocere to get formed through the excess of humidity created [9]; still, humidity in the external environment is not a compulsory condition for the occurrence of adipocere if the state of hydration of the

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body is big enough ante-mortem. This explains the presence of adipocere in many cases found in dry environment [1];

-presence of ground water [3];

-alkaline *p*H [4, 9];

-presence of anaerobic bacterial species, especially *Clostridium perfringens* [1, 4], which produces lecithinase, an enzyme that facilitates the hydrolyse and hydrogenation of fat [10];

-reduced level of oxygen [11];

-te increased temperature [4].

The individual factors involved are:

-state of hydration and the presence of the fat tissue-adipocere can vary even in the case of the same individual, depending on humidity and the quantity of fat tissue;

-presence of clothing with absorbent properties [4];

-level of sodium and potassium in the tissues [11].

There are some funeral practices that favour adipocere to get formed, such as: the particular construction of the coffin with a waterproof layer, used to prevent the leakage of liquid decomposition products, mattresses and clothing made of polyesters or the diapers [12].

Water elimination during the hydrolysis process and an increased acidity prevents the development and multiplication of bacteria, thus inhibiting the appearance of rotting [1].

Factors that delay or inhibit the formation of adipocere are:

-burial in a coffin;

-covering the body with synthetic materials (on the contrary, some authors [13] consider that this factor favours the formation of adipocere);

-low temperature;

-the aerobic environment [4, 13];

-the presence of calcium oxide/lime [4];

-the extreme pH: a very alkaline *p*H inhibits, and an acidic *p*H delays the process of saponification, possibly because of a low bacterial load [14].

In this paper the authors present a case in which they observed the partial conservation by saponification and make a synthesis of the data found in the literature which refer to this natural conservation process, insisting upon the chemical processes involved.

Experimental part

The body of a woman was found in November in a sewage collection basin located behind a hospital. The identification by teeth analysis and the clothes revealed that the corpse belonged to a 73 year-old woman who had been admitted to that hospital and who had disappeared in the month of July that year.

The necropsy showed: an advanced state of putrefaction and partial skeletonization, associated with areas of saponification, placed on the face, neck and thorax, the anterior wall of the abdomen, where the soft tissue still persists, having a moist aspect with a brownish yellowish white colour. The upper limbs persist partially, the tegument and muscles are coloured in brown yellowish white; the phalanges of the left hand are missing and parts of the phalanges from the right hand. It also showed a partial absence of the soft tissues of the anterior wall of the abdomen, leaving the abdominal organs and matte brown muscles visible.

The partial lack of soft parts in the lower limb in the thigh and calf allows the femur bone to be seen; the remaining parts have a whitish yellowish brown coloration, the muscles look matte brown; the left lower limb partially lacks the soft parts of the knee and calf, leaving the patella to be seen; the remaining parts are coloured in brownish white, yellowish and sometimes black, the muscles with a brownish matte aspect; the phalanges from both feet are missing.

The internal examination of the body showed the absence of the brain. Otherwise, the internal organs are present in a relative state of conservation: the chest muscles with wet saponification areas, of a whitish brown colour associated with blackish areas, parietal and visceral pleural are matte, whitish grey reddish, with partial adhesions in the form of whitish breeches; the lungs have a whitish black colour on the surface and also in section, look dry, without crepitus; the trachea and large bronchi with free lumen, the mucosa is a matte whitish black; the pericardium is whitish grey, with a free pericardial cavity; the heart has slightly increased dimensions (13×12×5 cm); the section of myocardium is brown with a few whitish streaks; the coronary arteries have thickened walls with whitish yellow deposits; the aorta is brownish red, with yellowish white inner coat and prominent atherosclerotic plaques through the lumen, the pulmonary artery has an integer inner coat, coloured in brownish white; peritoneum with a matte grey aspect, the peritoneal cavity is empty; the stomach has a brownish grey mucosa; the intestines look matte grey whitish yellow on the surface; the pancreas looks whitish yellow in section; the spleen has a matte grey capsule and is dark in section; the kidneys are matte brown, having a blurry cortical/medullary limit; the liver is brown and looks dry and like clay; the uterus is white and also white in the section, empty uterine cavity. The remaining skeleton is entirely intact.

Results and discussions

In this case, according to enquiry data, the victim disappeared during the month of July. Judging by the stage of cadaveric changes (advanced putrefaction associated with partial saponification) we can estimate that the death occurred 2-6 months before the body was found. The high temperature during the summer made the putrefaction process start rapidly, and also made saponification only partial, which made it impossible to identify the presence or absence of signs of violence in the soft tissues. However, it was possible to identify and to examine the internal organs (except the brain- as this organ is rapidly destroyed by putrefaction due to its increased water content). This showed the presence of cardiac pathological lesions: aortic-coronary atherosclerosis and myocardosclerosis [15].

Since it was first identified until the present day, many authors made numerous studies in order to analyse adipocere in detail, and also its chemical structure, its importance in forensic practice, but also in the social one, for example because it makes it impossible for people to reuse graves as the bodies are not decomposed [12].

Adipocere was simply defined in 1789 as *soap resulting from reactions of fat with ammonia* [4] and now we got to complex analysis, with a qualitative and quantitative identification of its compounds. Table 1 presents a synthesis of the results of studies on the chemical structure of adipocere made over the years.

The fat tissue of the human body is made primarily of triglycerides. During decomposing, hydrolysis of triglycerides will give rise to free fatty acids [7, 14] that are insoluble [4, 5] and to glycerol (fig. 1).

Subsequently, by hydrogenation, the free fatty acids will be converted into saturated fatty acids [17], the most abundant of which are myristic, palmitic, stearic [18] (fig. 2).

Year	Compounds	Author
1789	Soap resulting from reactions of fat with ammonia	den Dooren de Jong
1812	Mixture of margaric and oleic acids combined with yellow odorous substances containing lime, potash and nitrogenous matter	Chevreul
1847	75% palmitic acid, 25% stearic acid, 2-3% lime (in hog adipocere)	Gregory W.
1860	Palmitic and oleic acids; coally matter	Wetherill C.M.
1853-1854	Decomposition of the body fats or loss the glycerine, with the conversion of oleic acid to adipocere	Wetherill C.M.
1917	Palmitic, stearic, oleic, hidroxy stearic acids, stearin, palmitin	Ruttan and Marshall
1922	Decline in oleic acid, increase in free fatty acids	Goy S.
1977-1979	Separation and identification of two oxo fatty acids: 9-chloro-10-methoxy hexadecanoic and octadecanoic acid	Takatori T and Yamaoka A.
1983	10-hydroxy-12-octadecenoic acid	Takatori T et al.
1997	Epicoprostanol	Adachi et al.
2008	Saturated fatty acids: myristic, palmitic, stearic, 10-hydroxy stearic; Unsaturated fatty acids: palmitoleic, oleic, linoleic	Notter SJ et al.
2011	Decline of triglycerides and increase of unsaturated and saturated fatty acids, salts of fatty acids and hydroxy fatty acids	Forbes et al.

Table 1
TEMPORAL EVOLUTION OF THE RESEARCH ON COMPOSITION OF ADIPOCERE
[4, 5, 16]

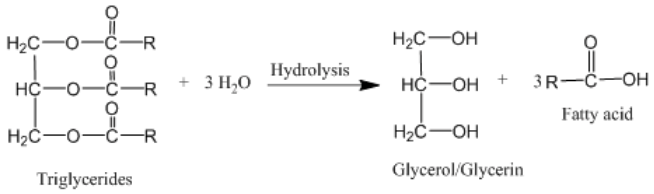

Fig. 1. Formation of glycerol and free fatty acids by hydrolysis of triglycerides

The proper environment conditions mentioned before-moist anaerobic environment [19], will lead to their transformation into adipocere [17]. With the passing of time, the glycerol will disappear [1]. Adipocere composition also includes unsaturated fatty acids (oleic and palmitoleic, depending on the degree of formation of adipocere) [20], fatty acid salts, hydroxy- and oxo-fatty acids [4, 19], which are less abundant [18] - between 3 and 20% of the total fatty acids [4]. Hydration and B-oxidation of the double bonds of oleic acid will result in an increased level of the palmitic acid, whereas their hydrogenation will result in the formation of the 10-hidroxistearic acid [7], which is insoluble, very characteristic to adipocere [4, 5]; the dehydrogenation of 10-hidroxistearic acid will form the oxo-stearic acid [7].

By comparing the composition of the adipocere with the one of the fresh subcutaneous fat, Adachi et al [16] identified for the first time a unique compound of adipocere: epicoprostanol, the reduced compound of cholesterol, suggesting that during the formation of adipocere there will be both oxidation processes and reduction ones.

The persistence of adipocere in the soil where the body was buried is given by the chemical characteristics of these saturated fatty acids: a high melting point (palmitic: 63 degrees, 10-hydroxy-stearic: 81 degrees) and a low hydro-solubility [18]. The palmitic, oleic and stearic acids, together with the glycerol, make up a matrix for what is left of the tissues, nerves and muscles, and thus the fat gets a certain resistance [21]. Although once it is formed, it can resist for tens or even hundreds of years [1], there are some factors that can degrade adipocere, like aerobic conditions and Gram positive bacteria [4].

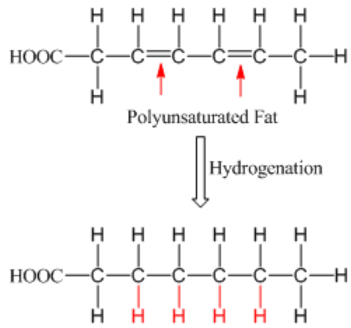

Fig. 2. Transformation of free fatty acids into saturated fatty acids by hydrogenation

Once formed, saponification may persist for tens or even hundreds of years [1], being relatively resistant to subsequent changes, preserving many soft tissues [9] when the environmental conditions are favourable. Because it inhibits putrefaction, adipocere allows the forensic identification of the body: the shape of the body is preserved, and in some cases the facial features (even if distorted) remain recognizable [1].

Adipocere may also preserve the injuries, helping in establishing the cause of death [18]; adipocere preserves very well for example the bullet holes [1].

Soil analysis is important for two reasons. The analysis of the soil where saponified body was found allows the assessment of the burying conditions that lead to the appearance of adipocere. The identification of adipocere constituents in the burying soil without the body being present suggests that it had been moved through a clandestine activity: the reduced pH of the soil and the reduced level of Ca were linked to adipocere in exchange for sodium and potassium ions, reduced microbial activity, high levels of P - which migrated from the body into the soil, organic C, cadaverine [4]. Considering the complexity of the soil samples, the often reduced quantity of the sample and of the chemical compounds searched, in order to get a realistic analysis that can offer accurate information, the sample must be prepared correctly before being analysed, as it cannot be introduced as such in the analysis instrument [22]. The problems raised by common techniques used to prepare the adipocere sample, i.e. thin-layer chromatography (TLC), liquid-liquid extraction (LLE) and column chromatography and also the excess of organic solvents used, the possible oxidation of polyunsaturated fatty acids through prolonged exposure to air require new studies to identify faster techniques, that will last very little time and that will not be influenced by the environment. In this respect, Kabir et al [22] underlines the usefulness of micro-sample preparation techniques that become more and more popular. A fast technique was created by Forbes et al., and it uses chloroform for extraction and hexamethylene disilazane (HMDS) for derivatization, making it possible to identify the individual esters through GC/MS [23, 24]. For isolating the free fatty acids from the neutral lipid components they created later a new method of solid-phase extraction (SPE), with amino propyl disposable cartridge columns [22].

Nowadays it is considered that the presence of adipocere makes it difficult or even impossible to estimate the post-mortem interval [4]. However, future researches might contribute to estimate this interval, or at least the interval in which the adipocere appeared, for example by using the analysis of epicoprostanol in relation to cholesterol, the ratio between them increasing with the post mortem interval increase [4, 16].

Dogs are often used in the forensic field because of their special olfactory skills. But if we want them to be really useful, they must be trained with appropriate chemical smells in order to be able to find the places where human bodies are buried. This way, chemical analysis of adipocere can identify the substances that can be used to train the dogs, considering the fact that they are most frequently used to find bodies before starting expensive digging. Among the volatile organic compounds in adipocere there are: pentanoic, butanoic, hexanoic acid, butanoic acid-butyl ester, hexanoic acid-ethyl ester, indole, dimethyl disulphide [17].

Conclusions

Saponification is a natural preservation process of the human cadavers if suitable environmental or individual conditions are fulfilled: increased humidity, ground water, low levels of oxygen and increased temperature, as well as the presence of important fat deposits and a high level of body hydration.

Gross appearance of adipocere may sometimes be helpful in identification of the face features or the injuries, but the combination with putrefaction- a very common possibility- makes more difficult the achievement of these goal. Adipocere also preserves pathological lesions, such as the sclerotic processes, as was the case presented in this paper, which made possible the establishment of the cause of death.

In addition, the chemical composition of adipocere proves very useful in forensic field when investigating clandestine activity or mass disaster scenes, as human remains detection dogs may be trained with the smell of its compounds or in the analysis of soil compounds when bodies are moved. Even though saponification- as well as the natural preservation processes in general- burden the estimation of post-mortem interval, new and thorough investigation of the chemical compounds may be useful in this issue, or at least in estimating the time since saponification process started.

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Manuscript received: 12.06.2017