Post-mortem volatiles of vertebrate tissue

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Abstract Volatile emission during vertebrate decay is a complex process that is understood incompletely. It depends on many factors. The main factor is the metabolism of the microbial species present inside and on the vertebrate. In this review, we combine the results from studies on volatile organic compounds (VOCs) detected during this decay process and those on the biochemical formation of VOCs in order to improve our understanding of the decay process. Microorganisms are the main producers of VOCs, which are by- or end-products of microbial metabolism. Many microbes are already present inside and on a vertebrate, and these can initiate microbial decay. In addition, microorganisms from the environment colonize the cadaver. The composition of microbial communities is complex, and communities of different species interact with each other in succession. In comparison to the complexity of the decay process, the resulting volatile pattern does show some consistency. Therefore, the possibility of an existence of a time-dependent core volatile pattern, which could be used for applications in areas such as forensics or food science, is discussed. Possible microbial interactions that might alter the process of decay are highlighted.

Keywords Post-mortem decay · Post-mortem volatiles · Decaying vertebrates · Volatile emission · Biochemical volatile formation · Volatiles of microbial metabolism

Introduction

A vertebrate is a complex structure of chemically interacting compounds. Some functional features like muscle contraction are constant in all vertebrates. Phospholipids in cell membranes, actin and myosin in muscle cells, or collagen and hydroxyapatite in bones are chemical structures that are indispensable in the biology of a vertebrate and exist in all vertebrates. The genetic makeup and diet have an impact on the quantitative chemical composition of a vertebrate. In muscle proteins, the relationship of different amino acids varies between species (Branscheid et al. 2007) and the quantitative composition of bones is influenced by both the diet (Sos et al. 1961) and species (Ravaglioli et al. 1996). The fat content, such as the composition and abundance of fatty acids, triglycerides, phospholipids, and steroids, shows high variability (Gurr 1988), and it is influenced by genetic makeup (Branscheid et al. 2007; Estevez et al. 2003; Insauti et al. 2005; Sink 1979a). The fat composition of the diet also directly influences the vertebrate’s fat composition (Lee et al. 2004; Sink 1979b). These results indicate that vertebrates contain chemical structures of low and high variability.

Consumers or destruents are heterotrophic organisms in the food chain that are able to feed on cadavers. Consumers are adapted to hunt vertebrate prey but can also feed on cadavers and, besides other sources, destruents can feed on dead vertebrates as well. A dead vertebrate has a complex and time-dependent faunal succession composed of birds (Moreno-Opo et al. 2010), omnivorous mammals (Haglund 1997), and insects (Amendt et al. 2004; Smith 1986). There exist very little experimental literature on the microbiological succession on dead vertebrates (Hopkins et al. 2000). Microorganisms are present in all the geographical regions of the world and play the important role of recyclers in ecosystems. They degrade organic matter to inorganic substances, which
are then made available to primary producers. The chemical reactions catalyzed by microbial enzymes yield volatile organic compounds (VOCs) as the by- or end-products. Micro-organisms can adapt their metabolism to their habitat. This adaption depends on many factors such as the complex and variable chemical composition of the decomposing dead vertebrate, temperature of the habitat, or competition between different microbial species (Kai et al. 2009). Consequently, the pattern of volatiles released during vertebrate decay can vary. The aim of this review is to summarize the volatiles whose presence has been consistently reported during the process of decay and to link these volatiles to their processes of formation. Based on the results, we discuss possible applications of these volatile patterns to forensics and the detection of food spoilage.

**Post-mortem decay initiated by endogenous enzymes**

Immediately after death, the heart activity ceases and the lack of oxygen leads to considerable physiological changes. Glycogen is degraded to lactic acid in the skeletal muscle (Branscheid et al. 2007). This decreases the pH during the first hours post-mortem. In the case of high muscle activity before death, this effect can occur even minutes after death because the muscle already lacks oxygen. Another consequence is the cessation of pyruvate transport across the mitochondrial membrane. The oxidation of pyruvate to acetyl-CoA stops, and acetyl-CoA cannot enter the citrate cycle. The respiratory chain requires the low electronegativity of oxygen because it generates the electron gradient necessary for ATP production. As ATP is a universal energy source in vertebrate cells, the termination of its anabolism leads to the cessation of regular metabolism. One of the consequences of low ATP levels is the binding of the myosin–actin complex in the muscle, resulting in rigor mortis. Another effect is the inability of the cells to repair their own membranes. Consequently, lysosomal hydrolytic enzymes are released in an uncontrolled fashion in vertebrate cells (Ito et al. 1991a, b). The proteolytic enzymes released include lysosomal cathepsins and calcium-dependent endopeptidases in the muscle cells. Endopeptidases mainly consist of calpain I for low calcium levels shortly after death and calpain II for high calcium levels. High calcium levels result from increasing influx due to the onset of decay of longitudinal tubuli in the muscle cells. These proteolytic enzymes fragment the myofibrils of muscle cells along the z-disk. They react with troponins T and I, tropomyosin, C-protein, filamin, vinculin, desmin, titin, and nebulin (Dransfield 1992; Goll et al. 1983; Koohmaraie 1992, 1994). Toldra (1998) reported lipase and phospholipase activity inside fermented ham, although halophilic microorganisms were only abundant on the surface. This suggested that the activity of lipolytic enzymes arose from the muscle tissue itself. Furthermore, lipoprotein lipase is present in the skeletal muscles of rats and hydrolyses di- and triglycerides of low-density lipoproteins at an optimum pH value of 8 to 9 (Miller et al. 1987; Motilva et al. 1993; Okuda 1991). Lysosomal acid lipase was found in the human liver, placenta, aorta, and leukocytes (Sando and Rosenbaum 1985), and it hydrolyses lipids at a pH optimum of 4 to 5 (Motilva et al. 1993; Negre et al. 1985). This suggests that the activity of lipoprotein lipase ceases shortly after death due to lactic acid accumulation. The low pH suppresses lipoprotein lipase and supports the activity of lysosomal acid lipase. The phospholipids of the cell membranes are degraded by phospholipases (Toldra 1998). Autolysis of arachidonic acid by the skin enzyme system of ayu fish (*Plecoglossus altivelis*) yields 2-octenal, 1-octen-3-ol, 2-nonenal, 2-octen-1-ol, and 3-nonen-1-ol, and the autolysis of eicopentaenoic acid yields 1,5-octadien-3-ol, 2,6-nonadienal, 2,5-octadien-1-ol, and 3,6-nonadien-1-ol (Zhang et al. 1992). In an anaerobic environment, fatty acids can remain in adipocere in certain circumstances. Several studies have examined adipocere formation under artificial (Forbes et al. 2005a, b, c; Yán et al. 2001) and field (Forbes et al. 2002; Gill–King 1999) conditions.

In general, lysosomal hydrolytic enzymes are active at an optimum temperature of 37 °C (Dutson 1983). Most of these enzymes are also active at lower temperatures but have lower activity. For example, some cathepsins are active at 25 °C, and Ca$^{2+}$-dependent proteinase (CAF) is responsible for proteolysis in meat stored at 0–4 °C (Goll et al. 1983). Another example is calpastatin, which inhibits calpains with 87% of the maximum rate at 25 °C and pH 7.5. Temperature and enzyme activity are correlated in a non-linear way (Goll et al. 1983).

Reactive oxygen species (ROS) are formed enzymatically, chemically, and photochemically. They react with lipids, amino acids, and sugars. Oxidized lipids release volatile aldehydes, alcohols, or alkenes. Oxidized methionine forms H$_2$S, while oxidized sugars form ketones (Choe and Min 2005). Living vertebrates compensate the cleavage reactions of ROS by repair processes or quench these highly reactive molecules by antioxidant defense mechanisms (Cadenas 1989). After death, these repair mechanisms cease, and ROS product emission increases. This increase might be the first sign of a post-mortem VOC pattern. During the active and advanced stages of decay, these emissions play a minor role in comparison to the volatiles produced by microbial activity.

**Post-mortem decay of vertebrates by micro-organisms**

The microbial decay of a vertebrate is a dynamic process that depends on a highly diverse assembly of microbial species,
their interactions (Kai et al. 2009; Stams 1994), and the climatic conditions of the habitat. Temperature has a strong effect on the microbial species composition. For instance, psychrotrophic or mesophilic bacteria exhibit optimal growth at different temperatures and also release different volatiles (Ercolini et al. 2009). Burial (Dent et al. 2004; Vass et al. 2008) and drowning or exposition (Dekeirsschieter et al. 2009; Statheropoulos et al. 2005) influence the microbial species composition. In general, the decay of a vertebrate is a process that occurs in different stages (Amendt et al. 2004; Dekeirsschieter et al. 2009) and can lead to complete decomposition or conservation, such as mummification. In the forensic literature, the decay of a vertebrate is often described in stages. The stage approach is a useful simplification of the decaying process as it is possible to characterize every stage of decay by its typical characteristics. Using this distinction, other phenomena like the infestation of the dead vertebrate with insect larvae can be described (Smith 1986). However, Matuszewski et al. found that a decomposing vertebrate can simultaneously exhibit different stages of decomposition (Matuszewski et al. 2010a) and Carter et al. states that the stages approach is an accepted and useful simplification but that the decaying process cannot be divided in discrete subunits (Carter et al. 2007). Therefore, most of the studies on volatiles of vertebrate decay (Table 3) don’t mention the stage of decay in which the volatiles occurred. Recently, alternative methods for correlating post-mortem interval (pmi) and decompositional process were proposed (Michaud and Moreau 2011).

Despite their destructive functions as destruents, microorganisms play an important role in living vertebrates by supporting the latter’s vital functions. The number of microorganisms in the human gut was estimated to be in excess of $10^{11}$ cells/g gut contents (Moore and Holdeman 1974). Many facultative or obligate anaerobic species such as Escherichia coli and Clostridium perfringens are present in the healthy intestine where they metabolize predigested nutrients entering from the stomach (Corry 1978). Micro-organisms can adapt their metabolism to the diet of the vertebrate. The relative amounts of ammonia emitted, fecal volatile sulfur compounds, phenol and urinary 4-methylphenol in human feces depend on the relative proportion of meat in the diet of the vertebrate (Cummings et al. 1979; Geypens et al. 1997). Intestinal micro-organisms prefer different substrates such as carbohydrates or amino acids as their nutrition source or depend on the metabolic products of other microbes (Smith and Macfarlane 1996). Various microbes are present on mucous membranes, sexual organs and the skin (Jenny 1983; Labows 1982; Straehlerpohl and Exner 1985). Yeasts and bacteria may be present ante-mortem in the lymphatic and vascular system as a result of infection or uptake during nutrition (Chang and Kollman 1989; Wells et al. 1988). After death, the micro-organisms present begin to colonize the body and disperse in an uncontrolled manner from their original location. The infestation of local tissues adjacent to the intestine and the capillaries of the lymphatic or vascular system characterize the beginning of this migration. A second important source of microbes is the mucous membrane of the respiratory system. This microbial community consists of Staphylococcus aureus, some Streptococcae, Neisseria catarrhalis, Corynebacterium xerosis, Coryne. pseudodiphtheriticum, the yeast species Candida albicans and many other microbial species. In guinea pigs, it was reported that the imunosystem could neutralize spreading microbes during the first 24 h after death (Gill et al. 1976). This led to the hypothesis that after death, ROS oxidation and autolysis dominate decay until the immunosystem fails and microbial growth increases. Of course, the number of microbes mentioned here is limited to only a few examples. For more detailed information, see Ercolini et al. (2009) and Corry (1978).

Carbohydrates

The main substrates for microbial growth offered by a vertebrate are water, carbohydrates, amino acids, fats and minerals (Dent et al. 2004). Carbohydrates are the most readily utilized nutrition source for both microbes and mammals (Boumba et al. 2008). The Embden-Meyerhof-Parnas (EMP) glycolytic pathway and the Entner–Doudoroff (ED) pathway yield pyruvate from glucose. Pyruvate is fermented to ethanol and acetic acid by bacteria and yeasts (Boumba et al. 2008; Gottschalk 1986). Other by-products of this fermentation are pyruvic acid, lactic acid, butanoic acid, propanoic acid, acetaldehyde, acetone, propan-1-ol, propan-2-ol, butan-1-ol and butane-1,3-diol (Boumba et al. 2008; Dent et al. 2004; Waksman and Starkey 1931). Incomplete aerobic degradation of sugar monomers by fungi yields organic acids such as glucuronic acid, citric acid and oxalic acid. Complete aerobic decay of sugar monomers results in carbon dioxide and water.

Clostridiaceae are obligate anaerobic bacteria that are present in the intestine and in anaerobic soil layers. Since they sporulate and can therefore survive under aerobic conditions, these bacteria can colonize a dead vertebrate from inside and from the external environment. This family is represented by species such as Clostridium perfringens (welchii), C. litus-eburense (Corry 1978; Haagsma 1991) and C. paraputrificum (Corry 1978). Many clostridiaceae species ferment pyruvate formed from carbohydrates to acetone, ethanol, butan-1-ol, acetic acid, butanoic acid or butane-1,3-diol (Boumba et al. 2008). Enterobacteriaceae such as E. coli are facultative anaerobes that are present in the intestine. E. coli ferments the pyruvate formed by glycolysis by mixed acid fermentation (Table 1) to lactic acid, succinic acid, acetic acid, formic acid and ethanol.
(Boumba et al. 2008). It can switch from glycolysis to lactose utilization to form pyruvate, which is then transformed to the volatile products acetic acid and ethanol. The genus *Klebsiella* of the family enterobacteriaceae is a gram-negative, facultative anaerobe that lives in soil, water or grain. *Klebsiella pneumoniae* subsp. *pneumoniae* is the only species of this genus known to be abundant in the intestine of vertebrates. Among intestinal enterobacteriaceae, this species is characterized by its use of butane-2,3-diol fermentation for energy production. Butane-2,3-diol, lactic acid, acetic acid, formic acid and ethanol are the main end-products of butane-2,3-diol fermentation (Gottschalk 1986). *Staphylococcus aureus* from the family Staphylococcaceae, formerly a member of Micrococcaceae, is a bacterium that is abundant in the soil, skin and respiratory system of vertebrates. Among intestinal enterobacteriaceae, this species is characterized by its use of butane-2,3-diol fermentation for energy production. Butane-2,3-diol, lactic acid, acetic acid, formic acid and ethanol are the main end-products of butane-2,3-diol fermentation (Gottschalk 1986). *Streptococcus faecalis* and *Enterococcus faecium* are present in the oral cavity, intestine, urethra, sexual organs and the main end-products of butane-2,3-diol fermentation (Gottschalk 1986). *Streptococcus faecalis* and *Enterococcus faecium* are present in the oral cavity, intestine, urethra, sexual organs and the main end-products of butane-2,3-diol fermentation (Gottschalk 1986). *Enterococcus faecalis* and *Enterococcus faecium* are present in the oral cavity, intestine, urethra, sexual organs and the main end-products of butane-2,3-diol fermentation (Gottschalk 1986). *Propionibacterium acnes* are aero-tolerant anaerobic species that grow relatively slowly on the skin and in the intestine of vertebrates. These bacteria can ferment carbohydrates or lactic acid to propanoic acid and acetic acid by propanoic acid fermentation (Table 1). Species of the family lactobacillaceae such as *Lactobacillus acidophilus*, which grows at a pH of 4–5 or lower, are facultative anaerobes or obligate anaerobes present in the intestine of vertebrates. They produce lactic acid (homofermentive) or lactic acid, acetate and ethanol (heterofermentative) by lactic acid fermentation. Bifidobacteriaceae such as *Bifidobacterium animalis* are anaerobic bacteria present in the intestine or sexual organs of vertebrates where they metabolize carbohydrates by the bifidum pathway to form lactic acid and acetic acid (Table 1). The contribution of yeasts to vertebrate decay is less than that of bacteria (Corry 1978). One yeast species reported to be present on decaying vertebrates is *Candida* sp., especially *Candida albicans* (Chang and Kollman 1989; Corry 1978), which is present in the intestine, on the mucous membranes of the oral cavity and the genitals and between fingers and toes. *Saccharomyces cerevisiae* (Corry 1978; Davis et al. 1972), another yeast species, is a facultative aerobe that obtains energy by respiration or fermentation. The constant internal putrefaction caused by these microorganisms and other microbes leads to the formation of organic and inorganic gases that cause bloating. The putrefaction of the cadaver that follows results in the breakage of the outer skin. Consequently, the inner fluid comes into contact with oxygen and aerobic species from the air and soil, which then colonize it. Aerobic bacillaceae species, mainly *Bacillus subtilis*, which is abundant in the upper soil layers, can adapt to facultative anaerobic conditions by switching to mixed acid fermentation of sugars. This microbe grows faster under aerobic conditions, but may switch to homofermentative lactic acid fermentation under anaerobic conditions. **Table 1** Products of carbohydrate-utilizing microbes in a facultative anaerobic environment

| Pathway                        | Microbial families and species | Location                          | Metabolic products                                      |
|--------------------------------|--------------------------------|-----------------------------------|--------------------------------------------------------|
| Mixed acid fermentation        | Enterobacteriaceae             | Intestine                         | Lactic acid, succinic acid, acetic acid, formic acid, ethanol |
|                                | *Escherichia coli*             |                                   |                                                        |
|                                | *Enterococcus faecalis*        |                                   |                                                        |
|                                | *Enterococcus faecium*         |                                   |                                                        |
|                                | *Bacillales*                   |                                   |                                                        |
|                                | *Bacillus subtilis*            |                                   |                                                        |
| Lactic acid fermentation       | *Streptococcus*                | Oral cavity, intestine, urethra, sexual organs | Lactic acid, succinic acid, acetic acid, formic acid, ethanol |
|                                | *Lactobacillus acidophilus*    | Intestine                         | Acetic acid, ethanol                                    |
| 2,3 Butanediol fermentation    | *Klebsiella pneumoniae* subsp. *pneumoniae* | Intestine                         | Butane-2,3-diol, lactic acid, acetic acid, formic acid, ethanol |
| Propanoic acid fermentation    | *Propionibacteriaceae*         | Intestine, skin                   | Propanoic acid, acetic acid                             |
| Bifidum pathway                | *Bifidobacteriaceae*           | Intestine, sexual organs          | Lactic acid, acetic acid                                |
|                                | *Bifidobacterium animalis*     |                                   |                                                        |
conditions. Pseudomonaceae are abundant in soil, water and on the skin of vertebrates. They are aerobic and cannot ferment. For energy production, they utilize the Entner–Doudoroff pathway, which yields pyruvate predominantly from hexoses. These microbes can also generate the electron gradient of the respiratory chain by reducing nitrate to nitrogen instead of reducing oxygen. Neisseriaceae such as Neisseria catarrhalis are aerobic species that are abundant on the mucosal membranes of vertebrates. Corynebacteriaceae are aerobic or facultative anaerobic bacteria, and several species such as Corynebacterium xerosis or Coryne. pseudodiphtheriticum inhabit the mucosal membranes or skin of vertebrates.

Amino acids

In all vertebrates, amino acids are the major components of muscle tissue proteins, membrane proteins and free proteins. Although the functional mechanisms of proteins are complex and diverse, they are composed of the same amino acids. Microbial proteases and peptidases yield free amino acids. Amino acid degradation can also lead to the formation of volatile products (Table 2).

Durlu-Özkaya et al. (Durlu-Özkaya et al. 2001) stated that putrescine, cadaverine, tyramine and histamine in the skeletal muscle are products of proteolytic bacterial metabolism and that E. coli plays an important role in the

| Table 2 Metabolic products of amino acid degradation |
|-----------------------------------------------------|
| Leucine                                             |
| Ehrlich pathway, anabolism                          |
| M. phenylpyruvica, S. xylosus, S. starnosus         |
| Propan-1-ol, 2-methyl-propan-1-ol, 2-methyl-butanol-1-ol, 3-methyl-butanol-1-ol, 3-methyl-butanal, 3-methyl-butanoic acid |
| Isoleucine                                          |
| Ehrlich pathway, anabolism                          |
| Yeast                                               |
| Propan-1-ol, 2-methyl-propan-1-ol, 2-methyl-butanol-1-ol, 3-methyl-butanol-1-ol, 3-methyl-butanoic acid |
| Threonine                                           |
| Yeast                                               |
| 1-Propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, 5-pentanol |
| Arginine > ornithine                                |
| Ornithine decarboxylase                             |
| Lysine decarboxylase                                |
| Tyrosine                                            |
| S. albus, B. fragilis, Fusobacterium sp., Bifidobacterium spp., C. paraputrificum, C. butyricum, C. sporogenes, C. septicum |
| E. coli, Proteus sp., E. faecalis, S. albus          |
| Phenyldalanine                                      |
| Phenylalanine decarboxylase + Fe^{3+}               |
| Pseudomonaceae (aerobe)                             |
| M. phenylpyruvica, S. xylosus                       |
| P. putida, E. coli, K. pneumoniae, B. halodurans    |
| Denitrifying bacteria                               |
| Tryptophan                                          |
| Bacteroides, Lactobacillus, Clostridium, Bifidobacterium spp., Peptostreptococcus |
| Cysteine                                            |
| Anaerobe                                            |
| Methionine                                          |
| Aerobe                                              |
| H. alvei, E. agglomeran, S. liquefaciens, A. putrefaciens and A. hydrophila |
| Methanethiol, dimethyl disulfide, dimethyl trisulfide, dimethyl tetrasulfide |
| Methanethiol, dimethyl sulfide                      |

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Decarboxylated lysine yields cadaverine (Gill-King 1999). The amino acids valine, leucine and isoleucine are fermented to higher alcohols such as propan-1-ol, 2-methyl-propan-1-ol, 2-methyl-butanol and 3-methyl-butanol by the Ehrlich pathway or in reverse as by-products of anabolic biosynthetic pathways (Boumba et al. 2008; Chen 1978; Derrick and Large 1993). Moraxella phenylpyruvica, Staphylococcus xylosus and Staphylococcus starnosus transforme degrade leucine to 3-methyl-butanol, 3-methyl-butanal and 3-methylbutanoic acid (Moller et al. 1998; Stahnke 1999). 4-Methylphenol and propanoic acid phenylester are products of anaerobic tyrosine catabolism by Staphylococcus albus, Bacteroides fragilis, Fusobacterium sp., Bifidobacterium spp., Clostridium paraputrificum, C. butyricum, C. sporogenes and C. septicum. Tyrosine degradation also yields 2-phenylethanol, phenylacetaldehyde and phenylacetic acid (Smith and Macfarlane 1996). E. coli, Proteus sp., Enterococcus faecalis and S. albus produce phenol under facultative anaerobic conditions from the same amino acid (Bone et al. 1976). Phenylalanine degradation yields 1-phenylethanol, 2-phenylethanol, phenylacetaldehyde, phenylacetic acid and phenylpropanoic acid (Luengo et al. 2001; Macfarlane and Allison 1986; Rabus and Heider 1998; Smith and Macfarlane 1996). Tryptophan degradation by Bacteroides, Lactobacillus, Clostridium, Bifidobacterium and Peptostreptococcus yields indole, indoyl acetic acid and indoyl propanoic acid (Smith and Macfarlane 1996). L-Phenylalanine can form greenish products in the presence of phenylalanine deaminase and ferre ions (Fe^{3+}) (Gill-King 1999). Ferre ions are present in the soil and hemoglobin. Hemolytic Streptococcus can degrade hemoglobin, leading to the accumulation of greenish products in the dead vertebrate. Under aerobic conditions, indolic and phenolic compounds are oxidized by monoo- and dioxygenases (Young and Rivera 1985). Under anaerobic conditions, these are fermented by methanogenic bacteria present in the large intestine to phenol, cyclohexanol, cyclohexanone, hexanoic acid, hexanedioic acid, butanoic acid, propanoic acid, acetic acid, CO2, H2 and methane (Young and Rivera 1985). Under aerobic conditions, Coccaceae, Pseudomonaceae, Bacteriaceae, Bacillaceae, Candida sp, Saccharomyces sp and certain higher fungi form pyruvate, acetyl-CoA and succinate. Possible by-products are quinoline, glutamate, 2-butyndioic acid, 2-oxopropanoic acid and acetic acid (Evans 1963). Yeasts can degrade the amino acids threonine, leucine, isoleucine and valine to 1-propanol, 2-methyl-butanol and 3-methyl-butanol (Boumba et al. 2008).

Sulfur compounds are prominent VOCs emitted by decaying vertebrates (Stensmyr et al. 2002). The sulfur-containing amino acids cysteine and methionine play an important role in the formation of volatile sulfur compounds such as hydrogen sulfide, dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, dimethyl tetrasulfide and thiois (Dent et al. 2004; Frenay 1967; Gill-King 1999; Kadota and Ishida 1972). The facultative anaerobic Proteus species from intestine, soil, and water releases hydrogen sulfide. Dimethyl sulfide is a product of methionine decomposition under aerobic conditions. Cysteine degradation can yield elemental sulfur under anaerobic conditions (Frenay 1967). Methanethiol is a product of methionine degradation (Frederick et al. 1957; Kadota and Ishida 1972; Wolle et al. 2006), and it can lead to the formation of dimethyl disulfide during GC analysis (Lestremau et al. 2004). This oxidative dimerisation may also occur during the proteolysis of vertebrates (Frederick et al. 1957). Methanethiol and dimethyl sulfide (Lindinger et al. 1998) were found to be by-products of the metabolism of Hafnia alvei, Enterobacter agglomeran, Serratia liquefaciens, Alteromonas putrefaciens and Aeromonas hydrophilia (Dainty et al. 1989). The amount of sulfur compounds emitted by bacteria is species-dependent (Stutz et al. 1991). Under aerobic conditions, some bacteria, such as those from the thiobacillus group, can transform sulfides to sulfurous acids, elemental sulfur, and sulfate (Dent et al. 2004). The formation of secondary products from sulfur compounds under natural and artificial conditions is an indicator of the reactivity of these compounds (Lestremau et al. 2004).

The variability of biogenic amines is high and depends on the micro-organisms growing on the tissue (Ansorena et al. 2002). Dimethylamine (Lindinger et al. 1998) or trimethylamine is formed by the oxidative decarboxylation of free amino acids by Sherwanella putrefaciens (Dainty et al. 1989; Lopez-Caballero et al. 2001). As these amines are not volatile under moderate temperature conditions, further degradation is necessary for the production of VOCs. In general, these compounds are further degraded to ammonia, oxidized to nitrite by Nitrosomonas spp. and further to nitrate by Nitrobacter spp. in the soil under aerobic conditions (Waksman and Starkey 1931). Aerobic Pseudomonaceae are proteolytic bacteria that use proline, glutamate, lysine or L-phenylalanine as their C and N sources (Herrera et al. 2010).

Facultative or obligate aerobic Bacillaceae excrete the non-specific proteolytic enzyme subtilisin (Higgins and Burns 1975). Micrococaceae such as Micrococcus luteus are aerobic and abundant in the soil and surface water and are also present on the skin of vertebrates. The proteolytic species Serratia proteamaculans, Pseudomonas fragi and Carnobacterium maltaromaticum were detected on beef (Ercolini et al. 2009). Other micro-organisms reported to grow on muscle tissue are the facultative anaerobe Brochothrix thermophaexa and the facultative anaerobe and aero-tolerant Leuconostoc mesenteroides, Leuc. curvatus, Pseudomonas lundensis and Pseudomonas fluorescens biovar. Moraxella species were also identified (Dainty et al. 1985; Stutz et al. 1991; Vanderzant et al. 1986). These
species are generally abundant in the respiratory tract (Molin and Ternstrom 1986).

Lipids

In vertebrates, all cells contain fat in their phospholipid membranes. Fatty acids such as linoleic acid or arachidonic acid are typically present in abundance in lipid membranes (Frankel 1983). Intramuscular fat consists of fat cells inside the muscular tissue (Gandemer 2002), and depot fat is the fat under the skin. Intramuscular fat and depot fat contain triglycerides. The hydrolysis of triglycerides by microbial lipolytic enzymes yields glycerol and fatty acids.

Glycerol degradation results in the formation of pyruvate, ATP and NADH. Pyruvate degradation yields products such as butan-1-ol, butanoic acid, acetone, ethanol, propionic acid, acetic acid, formic acid, lactic acid, propane-1,3-diol, propene-1,2-diol and butane-2,3-diol (Boumba et al. 2008). Many Clostridiaceae are obligate anaerobic species that ferment pyruvate to acetone, ethanol, butan-1-ol, acetic acid, butanoic acid or butane-1,3-diol. E. coli catabolizes glycerol to propanol and propanoic acid (Boumba et al. 2008).

The fatty acids of vertebrates are further oxidized or hydrogenised (Dent et al. 2004) to several volatile compounds. However, there is a lack of detailed studies on volatile formation by microbial fatty acid degradation (Combet et al. 2006; Kionka and Kunau 1985). Although the beta-oxidation enzymes of prokaryotes, fungi, and plants differ from each other (Heath et al. 2002; Kionka and Kunau 1985), their catabolic activity is comparable and yields acetyl-CoA or propionyl-CoA (Gottschalk 1986). Acetyl-CoA is degraded to either ethanol, acetic acid, acetaldehyde, and acetone or to propan-1-ol, propan-2-ol, and propionic acid (Boumba et al. 2008). The volatile by-products of lipoxygenase activity in fungi are mainly C6 or C9 aldehydes and C8 alcohols or C8 ketones (Combet et al. 2006; Feussner et al. 1997).

Aerobic oxidation of linoleic acid yields 2,4-decadienal, 3-nonenal, hexanal, 2,4,7-deca trien al, 2,6-nonadienal, 3-hexenal, and propanal. Further chemical groups include ketones, hydrocarbons, acids, and epoxides. C. perfringens (welchii) (Polson et al. 1985) or other species from the genera Pseudomonas, Acinetobacter, and Bacillus (Gottschalk 1986) are lipolytic and yield volatile aldehydes, ketones, or esters (Boumba et al. 2008; Dent et al. 2004). Oxidation of linoleic acid in fungi yields the eight-carbon volatiles 1-octen-3-ol, octan-3-ol, octan-1-ol, 1-octen-3-one, and octan-3-one (Combet et al. 2006). Tetradecanoic acid, hexadecanoic acid, octadecanoic acid, 9-octadecenoic acid, 10-hydroxyoctadecanoic acid, and (Z, Z)-9,12-octadecadienoic acid are part of the adipocere. The latter can form in the later stages of decay by saponification of fatty acids under anaerobic conditions, neutral or slightly alkaline pH, and in the presence of certain enzymes (Forbes et al. 2002, 2005a, b, c; Gill-King 1999; Notter et al. 2009).

Bones

Bones consist of organic and mineral components. The organic components are mainly fat and proteins. In ancient bones, it is possible to detect triglycerides, free fatty acids, collagen, amino acids, and non-collagenous proteins such as osteocalcin and haemoglobins (Evershed et al. 1995; Schultz 2006; Vanklinken et al. 1994; Vanklinken and Hedges 1992). Therefore, fat degradation can lead to the release of volatiles in the later stages of decay. Fat is the major component of marrow consisting of different saturated and unsaturated fatty acids, omega-6 polyunsaturated fatty acid (PUFA), and omega-3 PUFA (Yeung et al. 2008). Vass et al. investigated the volatile emissions of exposed mammalian bones. They found different ratios of ketones, aldehydes, and alcohols in the four different mammalian species, and among other compounds they identified nonan-2-one, nonanal, hexanal, and hexan-1-ol (Vass et al. 2008). Ketones and aldehydes are products of fat degradation (Boumba et al. 2008; Dent et al. 2004). Alcohols, especially hexan-1-ol and 1-octen-3-ol, are by-products of fungal metabolism (Borjesson et al. 1993; Korpi et al. 1998; Larsen and Frisvad 1995; Pasanen et al. 1996; Sunesson et al. 1996; Thakeow et al. 2008), and signs of fungal activity are present on bones (Marinho et al. 2006). ROS might also play a role in the formation of volatiles from bones (Choe and Min 2005). Of the total bone proteins, 90% to 95% consist of different collagen proteins. These are degraded to peptides by bacterial collagenases (Macfarlane and Allison 1986). Streptomyces spp. are aerobic bacteria living in the soil, and they can cleave the strong disulfide bonds in the collagen molecule (Gray and Williams 1971). The mineral component of bones consists of hydroxyapatite, which is degraded by physical weathering.

Microbial interactions

Micro-organisms can show ecological interactions such as mutualism or competition. These interactions affect the pattern of volatiles released by the decaying vertebrate. Smith et al. investigated the formation of phenolic and indolic compounds by intestinal microbes in feces under anaerobic conditions and found variations in the relative quantity of phenolic and indolic volatiles, depending on the microbial density (Smith and Macfarlane 1996). Therefore, microbial density seems to influence their metabolic pathways due to inter- and intraspecific interactions. An example of mutualism is the fermentation of ethanol to acetate and H2. As the H2 concentration increases, the growth of the ethanol-
fermenting microbes decreases. The presence of microbes that utilize $H_2$ to reduce $CO_2$ to methane increases the growth rate of the first micro-organism (Bryant et al. 1967). The suppression of microbial ethanol fermentation in the presence of hydrogen-utilizing micro-organisms is an example of the competition between microbes (Iannotti et al. 1973). The lower partial pressure of $H_2$ leads to its formation from NADH by ethanol-fermenting microbes instead of coupling of the oxidation of NADH to the reduction of acetyl-CoA to ethanol. Both mechanisms may be important for post-mortem ethanol detection. The mycelial growth and spore germination of fungi are inhibited by $P. fluorescens$ and other Pseudomonaceae (Fernando et al. 2005). Dimethyl trisulfide is one of the inhibitory substances utilized by the bacteria in this competitive interaction. Phenol is an antiseptic substance emitted by proteolytic Bacillaceae (Gu et al. 2007), $E. coli$, Proteus sp., Enterococcus faecalis and $S. albus$ (Bone et al. 1976), and it might constrain the growth of other microbes. $Trichosporon cutaneum$ can degrade phenol, but the presence of cresols such as 4-methylphenol delays this reaction (Alexieva et al. 2008). 4-Methylenphenol is a metabolic product of $S. albus$, $B. fragilis$, Fusobacterium sp., Bifidobacterium spp., $C. paraputtrificum$, $C. butyricum$, $C. sporogens$, and $C. septicum$. All these data suggest a complex interaction based on phenolic compounds. The lipid metabolism of microbes yields fatty acids that show an antimicrobial effect (Jay 2000). In particular, the emission of aldehydes increases during the later stages of decay (Dekeirsschieter et al. 2009; Vass et al. 2008). This might lead to the conclusion that fatty acids are prominently decomposed in the later stages of decay to form aldehydes and that interactions based on fatty acids might occur during these stages. Volatiles emitted by the environment can also influence microbial growth on decaying vertebrates (Gilbert et al. 1969). For instance, thiols and sulfides were found to stimulate (King and Coley-Smith 1969) or suppress fungal growth (Lewis and Papavizas 1972). These results show the complexity of microbial interactions, which affect the VOCs emitted.

Post-mortem volatiles

The results of volatile analysis are influenced by abiotic factors, sampling technique, and analytical setup. Abiotic factors directly influence the release of volatiles from a vertebrate. As mentioned above, the temperature influences the activity of volatile-generating enzymes and the microbial species composition. Moisture also affects the process.

The compounds identified by volatile analysis depend on the sampling technique and the analytical setup. The sampling technique can be passive, for instance, in a closed container, or active with a constant gas flow. Depending on the exposed adsorbent, the compounds enrich according to their chemical properties. A non-specific material adsorbs many different volatiles, whereas a specific material is selective for characteristics such as polarity or certain functional groups. As an example, the SPME (Solid Phase Micro Extraction) technique was developed for uncomplicated and fast volatile sampling in a static headspace. A short fiber covered with an adsorption material is exposed to the headspace of the sample. After equilibration between the solid and the gaseous phase, the fiber is directly inserted in the injector of a GC system and the volatiles are thermally desorbed for analysis. The surface of the SPME fibers is modified to adsorb different classes of volatiles. For instance, polydimethylsiloxane (PDMS)-covered fibers adsorb predominantly non-polar compounds or Carbowax-covered fibers adsorb predominantly polar compounds. There exist several surface adsorbents, which can be combined with each other in order to achieve selectivity or non-selectivity during sampling. Some fiber types used in analytical studies are listed in Table 4. The exposure time of the fiber is important as well because a short exposure time favors the adsorption of compounds with a high volatility, while discriminating compounds with a low volatility. A long exposure will lead to desorption of highly volatile compounds, while additional low-volatility compounds are adsorbed. Moreover, individual adsorption properties may vary between fibers from different lots. Therefore, quantification with the SPME technique requires an internal standard with known quantity. SPME can be used for the extraction of compounds from liquid solutions (Ercolini et al. 2009). The liquid extracts can be esterified to enhance the trace analysis (Forbes et al. 2002, 2005a, b, c). For more information on SPME sampling, see Tholl et al. (2006). Dynamic headspace sampling techniques are used to find volatiles with low emission rates. A continuous air stream is led through an adsorbent to trap the volatiles out of the sampling system. Either the air is circulated in the sampling system or filtered air enters the system, is enriched with volatiles from the sample, and led through the adsorbent and then out of the system. For dynamic headspace sampling, different adsorbents can be used. Tubes filled with different selective or unselective adsorbent powders, for instance, TENAX, Carbetrap, Carbtrap C, and Carbosieve S-III, are commercially available. For increasing performance, a tube can be filled with different adsorbent powders. Vass et al. used triple-sorbent traps for their analysis of buried human remains (Vass et al. 2004, 2008). Another possibility is the trapping of the volatiles by directing the gas flow through cooled liquid solvents such as cyclohexane, methanol, or dichloromethane. Tholl et al. (2006) give a more detailed description of volatile sampling techniques (Tholl et al. 2006).

Any chemical analysis of the adsorbed volatile compounds has its own analytical window that enables the
identification of certain volatiles while excluding others. For instance, the polarity of a compound influences its retention on a GC column. A low molecular weight polar compound might only be detectable by high retention on a polar column and might be undetectable because of lower retention on a non-polar column. Furthermore, the detection of stereo isomers requires enantiomer-selective columns retention on a non-polar column. Furthermore, the detection of stereo isomers requires enantiomer-selective columns with modified surfaces (Dotterl et al. 2006). The identification of isotope ratios, like $^{13}$C/$^{12}$C or $^{15}$N/$^{14}$N, requires a modified GC setup (Boschker and Middelburg 2002; Meier-Augenstein 2002). Both techniques can be used to specify trace analytical results. Especially, the identification of isotope ratios in cadaveric volatile compounds can give important information on the nutrition of the dead vertebrate or the microbial communities growing on it (Boschker and Middelburg 2002; Meier-Augenstein 2002).

Finally, the detector attached to the column determines the information collected on the substances. A flame ionization detector (FID) gives information about the retention time and the peak area of compounds in a sample. Identification and quantification can only be achieved by using standard compound samples with known concentration. However, the selection of standard compounds is problematic when the compound to be identified is unknown. A mass spectrometer (MS) is able to identify compounds not only by the retention time of standard compounds but also by comparing the fragment pattern of a compound in a sample with a fragment pattern database. However, a standard compound sample has to be used for exact identification as well because the fragment pattern database is restricted to some thousand compounds and several compounds have the same fragment pattern. For quantification, a reference quantity is necessary as well. The selection of possible standard compounds is much easier with a MS system than with a FID because the database gives a pre-selection of possible candidates. However, a false identification is still possible when two compounds have very similar mass spectra and the same retention time. In order to improve the trace analysis, it is possible to use two-dimensional gas chromatography (GC×GC). Two columns with different polarity are coupled to each other and, as a result, every compound has two retention indices. Two compounds, which have the same retention time on a non-polar column, might have a different retention time on the second polar column in a two-dimensional GC. Another approach to enhance compound identification is the time of flight MS (TOFMS). This detector measures the fragment-specific flight time from the point of fragmentation to the detector plate. TOFMS analysis allows a more precise differentiation between atomic masses in comparison to conventional mass spectrometers. The studies listed in Table 4 use GC/MS analysis; only Kalinova et al. is an exception due to using GC×GC-TOFMS (Kalinova et al. 2009). Hübschmann et al. give further information on gas chromatography and coupled detector systems (Hübschmann 2009). Since it is known that the analytical method influences the results, Table 4 has been provided to supplement Table 3 and list selected information on sampling, sample preparation, and sample analysis. Another factor in chemical analysis is the modification of volatile compounds during the analytical procedure. For instance, volatile sulfur compounds can be transformed during GC-MS analysis (Lestremau et al. 2004). The analysis of post-mortem volatiles does not only depend on the analytical parameters itself. Other decomposers might influence the volatile pattern of a decaying vertebrate by feeding. Scratches or holes made by scavengers can open the dead body before it breaks up due to swelling. As a result, oxygen will infiltrate the body, favoring the growth of aerobic or facultative aerobic microbial species and suppressing that of obligate anaerobes. Aerobic (Straehlerpohl and Exner 1985) and anaerobic (Gulzow 1982) micro-organisms of the scavengers oral cavities could be introduced. Larval growth might influence the volatile pattern because proteolytic enzymes are excreted by the larvae. The cuticle hydrocarbons of these insects might also contribute to the volatile pattern. Burying beetles (Nicrophorus spp.) even bury small vertebrae and inhibit the decay with excretions from their glands (Kalinova et al. 2009).

Table 3 lists the VOCs reported in studies on the decay of vertebrate tissue. Because of the large number of reported VOCs, the list contains only those that have been identified in more than one study. The volatile compounds butane-1,2-diol, butane-1,3-diol, propane-1,3-diol, propane-1,2-diol, butane-2,3-diol, propan-1-ol, and propan-2-ol are not reported in any of the reviewed experimental studies although they are known to be volatile products of amino acid degradation (Boumba et al. 2008). Table 4 contains additional information about the method used.

### Application of post-mortem volatiles

Insects are important cues for criminal investigations as their larval development on corpses can help to estimate the time since death. It is not possible to determine the exact day of death as larval development is influenced by many environmental factors. Therefore, additional cues are necessary to support juristically accepted estimation of the pmi. Several authors suggested the use of insects to select volatiles, which are important in the dynamic emission pattern of vertebrate decay (LeBlanc and Logan 2010; Paczkowski et al. 2011; Statheropoulos 2005; Vass et al. 2002). These volatiles could be an additional cue for determining the time since death. However, the variation of cadaveric volatiles is very high. In order to find useful
| Compound          | CAS number | Origin                                      | Publication                                      |
|-------------------|------------|---------------------------------------------|-------------------------------------------------|
| **Alcohols**      |            |                                             |                                                 |
| Ethanol           | 64-17-5    | Carbohydrates, amino acids, lipids         | (Gottschalk 1986; Corry 1978; O’Neal and Poklis 1996; Stutz et al. 1991; Dent et al. 2004; Statheropoulos et al. 2005; Statheropoulos et al. 2007; Boumba et al. 2008; Dekeirsschieter et al. 2009) |
| 2-Methyl-propan-1-ol | 78-83-1    | (Derrick and Large 1993; O’Neal and Poklis 1996; Statheropoulos et al. 2005; Boumba et al. 2008; Dekeirsschieter et al. 2009) |
| Butan-1-ol        | 71-36-3    | Carbohydrates, lipids                       | (O’Neal and Poklis 1996; Gill-King 1999; Dent et al. 2004; Statheropoulos et al. 2005; Boumba et al. 2008; Dekeirsschieter et al. 2009) |
| Butan-2-ol        | 78-92-2    | Alkanes                                    | (O’Neal and Poklis 1996; Dekeirsschieter et al. 2009) |
| 2-Methyl-butanol-1-ol | 137-32-6  | Carbohydrates                              | (Derrick and Large 1993; Boumba et al. 2008)     |
| 3-Methyl-butanol-1-ol | 123-51-3   | (Boumba et al. 2008)                       |                                                 |
| Pentan-1-ol       | 71-41-0    | Threonine, leucine, isoleucine, valine      | (Lorenzo et al. 2003; Statheropoulos et al. 2005; Vass et al. 2008; Boumba et al. 2008; Dekeirsschieter et al. 2009; Hoffman et al. 2009) |
| Hexan-1-ol        | 111-27-3   | Fungal metabolism                          | (Stutz et al. 1991; Haze et al. 2001; Statheropoulos et al. 2005; Hoffman et al. 2009) |
| 2-Ethyl-hexan-1-ol | 104-76-7   |                                             | (Vass et al. 2004; Statheropoulos et al. 2007; Dekeirsschieter et al. 2009; Ercolini et al. 2009; Hoffman et al. 2009) |
| **Acids**         |            |                                             |                                                 |
| Formic acid       | 64-18-6    | Glycerol, pyruvate                         | (Gottschalk 1986; Dekeirsschieter et al. 2009) |
| Acetic acid       | 64-19-7    | Carbohydrates, indolic/phenolic compounds, glycerol | (Gottschalk 1986; Dent et al. 2004; Boumba et al. 2008) |
| Propanoic acid    | 79-09-4    | Amino acids                                | (Gill-King 1999; Statheropoulos et al. 2005; Boumba et al. 2008; Dekeirsschieter et al. 2009; Hoffman et al. 2009) |
| Compound                        | CAS number | Origin                                      | Publication                                                                 |
|--------------------------------|------------|---------------------------------------------|----------------------------------------------------------------------------|
| Butanoic acid                  | 107-92-6   | Carbohydrates, lipids                       | (Gill-King 1999; Lorenzo et al. 2003; Dent et al. 2004; Statheropoulos et al. 2005; Boumba et al. 2008; Dekeirsschiet et al. 2009; Hoffman et al. 2009) |
|                                |            | (Boumba et al. 2008), amino acids            |                                                                            |
|                                |            | (Demeyer and Vannevel 1979)                 |                                                                            |
| Pentanoic acid                 | 109-52-4   | Amino acids                                 | (Lorenzo et al. 2003; Dekeirsschiet et al. 2009; Hoffman et al. 2009)      |
|                                |            | (Demeyer and Vannevel 1979)                 |                                                                            |
| Hexanoic acid                  | 142-62-1   | Indolic/phenolic compounds                   | (Lorenzo et al. 2003; Dekeirsschiet et al. 2009; Hoffman et al. 2009)      |
|                                |            | (Young and Rivera 1985)                     |                                                                            |
| Tetradecanoic acid             | 544-63-8   | Lipids (Gill-King 1999)                     | (Forbes et al. 2002, 2005a, b, c; Notter et al. 2009)                      |
|                                |            |                                              | 11,12,13,14                                                                |
|                                |            |                                              |                                                                            |
| Octadecanoic acid              | 57-11-4    | Lipids (Gill-King 1999)                     | (Forbes et al. 2002, 2005a, b, c; Notter et al. 2009)                      |
|                                |            |                                              | 11,12,13,14                                                                |
|                                |            |                                              |                                                                            |
| 9-Octadecenoic acid            | 112-80-1   | Lipids (Gill-King 1999)                     | (Yan et al. 2001; Forbes et al. 2002, 2005a, b, c; Notter et al. 2009)    |
|                                |            |                                              | 10,11,12,13,14,15                                                          |
|                                |            |                                              |                                                                            |
| (Z, Z)-9,12-Octadecadienoic acid| 60-33-3    | Lipids (Gill-King 1999)                     | (Nushida et al. 2008; Notter et al. 2009)                                   |
|                                |            |                                              | 15,16                                                                     |
| 10-Hydroxyoctadecanoic acid    | 638-26-6   | Lipids (Gill-King 1999)                     | (Forbes et al. 2002; Forbes et al. 2005a, b, c)                            |
|                                |            |                                              | 11,12,13,14                                                                |
|                                |            |                                              |                                                                            |
| Esters                         |            |                                              |                                                                            |
| Acetic acid propyl ester       | 109-60-4   |                                              | (Statheropoulos et al. 2005; Dekeirsschiet et al. 2009)                    |
| Butanoic acid ethyl ester      | 105-54-4   |                                              | (Statheropoulos et al. 2005; Hoffman et al. 2009)                          |
| Butanoic acid butyl ester      | 109-21-7   |                                              | (Lorenzo et al. 2003; Dekeirsschiet et al. 2009; Hoffman et al. 2009)      |
|                                |            |                                              | 1,2,3,8                                                                    |
| Aldehydes                      |            |                                              |                                                                            |
| Butanal                        | 123-72-8   | Alkanes (Arp 1999)                          | (Stutz et al. 1991; Vass et al. 2008; Dekeirsschiet et al. 2009)           |
|                                |            |                                              | 1,2,3,5,17                                                                 |
| Pentanal                       | 110-62-3   | Lipids (Boumba et al. 2008)                 | (Statheropoulos et al. 2005; Vass et al. 2008; Dekeirsschiet et al. 2009) |
|                                |            |                                              | 1,2,3,5,6                                                                  |
| Hexanal                        | 66-25-1    | Lipids (Boumba et al. 2008)                 | (Haze et al. 2001; Lorenzo et al. 2003; Statheropoulos et al. 2005; Hoffman et al. 2009; Ercolini et al. 2009) |
|                                |            |                                              | 9,18                                                                      |
| Heptanal                       | 111-71-7   | Lipids (Boumba et al. 2008)                 | (Stutz et al. 1991; Haze et al. 2001; Lorenzo et al. 2003; Vass et al. 2008; Dekeirsschiet et al. 2009; Hoffman et al. 2009) |
|                                |            |                                              | 1,2,3,5,9,17                                                               |
| Nonanal                        | 124-19-6   | Lipids (Boumba et al. 2008)                 | (Haze et al. 2001; Vass et al. 2004; Vass et al. 2008; Ercolini et al. 2009; Hoffman et al. 2009) |
|                                |            |                                              | 5,8,9,18                                                                  |
| Decanal                        | 112-31-2   |                                              | (Vass et al. 2004; Vass et al. 2008)                                       |
|                                |            |                                              | 4,5                                                                       |
| Ketones                        |            |                                              |                                                                            |
| Acetone                        | 67-64-1    | Carbohydrates                               | (Stutz et al. 1991; O’Neal and Poklis 1996; Gill-King 1999; Vass et al. 2004; Dent et al. 2004; Statheropoulos et al. 2005; Statheropoulos et al. 2007; Boumba et al. 2008; Dekeirsschiet et al. 2009) |
|                                |            | (Boumba et al. 2008)                        |                                                                            |
| Butan-2-one                    | 78-93-3    | Alkanes (Patel et al. 1979)                 | (Stutz et al. 1991; Statheropoulos et al. 2005; Statheropoulos et al. 2007; Dekeirsschiet et al. 2009) |
|                                |            |                                              | 1,6,7,17                                                                  |
| Pentan-2-one                   | 107-87-9   | Alkanes (Patel et al. 1979)                 | (Statheropoulos et al. 2005; Dekeirsschiet et al. 2009)                    |
|                                |            |                                              | 1,2,3,6                                                                    |
| Cyclohexanone                  | 108-94-1   | Indolic/phenolic compounds                   | (Statheropoulos et al. 2005; Hoffman et al. 2009)                          |
|                                |            | (Young and Rivera 1985)                     |                                                                            |
| Compound                      | CAS number | Origin                  | Publication                                                                 |
|-------------------------------|------------|-------------------------|-----------------------------------------------------------------------------|
| Heptan-2-one                  | 110-43-0   |                         | (Statheropoulos et al. 2005; Dekeirsschieter et al. 2009; Hoffman et al. 2009) |
| Nonan-2-one                   | 821-55-6   | Lipids                  | (Statheropoulos et al. 2007; Vass et al. 2008; Dekeirsschieter et al. 2009; Ercolini et al. 2009) |
| Homoaromatic compounds        |            |                         |                                                                             |
| Benzene                       | 71-43-2    |                         | (Statheropoulos et al. 2005; Vass et al. 2008)5,6                          |
| Methylbenzene                 | 108-88-3   |                         | (Stutz et al. 1991; Vass et al. 2004; Statheropoulos et al. 2005; Statheropoulos et al. 2007; Vass et al. 2008; Hoffman et al. 2009)4,5,6,7,8,17 |
| 1,2-Dimethylbenzene           | 95-47-6    |                         | (Statheropoulos et al. 2007; Vass et al. 2008)5,7                          |
| 1,3-Dimethylbenzene           | 108-38-3   |                         | (Statheropoulos et al. 2005; Statheropoulos et al. 2007)5,7                |
| 1,4 Dimethylbenzene           | 106-42-3   |                         | (Vass et al. 2004; Statheropoulos et al. 2005; Statheropoulos et al. 2007; Vass et al. 2008; Hoffman et al. 2009)4,5,6,7,8 |
| 1,2,3-Trimethylbenzene         | 526-73-8   |                         | (Statheropoulos et al. 2005; Statheropoulos et al. 2007)6,7                |
| Ethylbenzene                  | 100-41-4   | Phenylalanine           | (Vass et al. 2004; Statheropoulos et al. 2005; Vass et al. 2008)4,6         |
| Ethynylbenzene                | 100-42-5   | Phenylalanine           | (Vass et al. 2004; Statheropoulos et al. 2007; Vass et al. 2008)5,6         |
| 1-Methyl-2-ethylbenzene       | 611-14-3   |                         | (Vass et al. 2004; Statheropoulos et al. 2005; Vass et al. 2008)4,5,6,7,8,17 |
| 2-Phenyl-propan-2-ol          | 617-94-7   |                         | (Vass et al. 2004; Vass et al. 2008)4,5                                   |
| Phenol                        | 108-95-2   | Tyrosine                | (Cummings et al. 1979; Statheropoulos et al. 2007; Vass et al. 2008; Dekeirsschieter et al. 2009)1,2,3,5,7,8 |
| 4-Methylphenol                | 106-44-5   | Tyrosine                | (Geypens et al. 1997; Statheropoulos et al. 2007; Dekeirsschieter et al. 2009)1,2,3,7 |
| 2-Phenylethanol               | 60-12-8    | Phenylalanine           | (O'Neal and Poklis 1996; Dekeirsschieter et al. 2009)1                      |
| Benzaldehyde                  | 100-52-7   | Phenylalanine           | (Stutz et al. 1991; Lorenzo et al. 2003; Vass et al. 2004; Dekeirsschieter et al. 2009; Hoffman et al. 2009)1,2,4,8,17 |
| Acetophenone                  | 98-86-2    | Phenylalanine           | (Statheropoulos et al. 2007; Dekeirsschieter et al. 2009)1,2,7             |
| 1-Methoxy-propylbenzene       | 59588-12-4 |                         | (Vass et al. 2004; Vass et al. 2008)4,5                                     |
| Naphthalene                   | 91-20-3    |                         | (Vass et al. 2004; Statheropoulos et al. 2007; Vass et al. 2008)3,5,7       |
| Nitrogen compounds            |            |                         |                                                                             |
| Trimethylamine                | 75-50-3    | Amino acids             | (Dainty et al. 1989; Stutz et al. 1991; Dekeirsschieter et al. 2009)1,2,3,7 |
| Benzonitrile                  | 100-47-0   | L-Phenylalanine         | (Vass et al. 2004; Dekeirsschieter et al. 2009)1,2,4                         |
| Indole                        | 120-72-9   | Tryptophan              | (O'Neal and Poklis 1996; Gill-King 1999; Vass et al. 2002; Lorenzo et al. 2003; Dent et al. 2004; Statheropoulos et al. 2005; Hoffman et al. 2009; Dekeirsschieter et al. 2009)3,6,8 |
marker volatiles, which are correlated to the time since death, it is possible to use insects as an information filter. Many insects have adapted their olfaction over millions of years to specific cadaveric volatiles (Huotari and Mela 1996; Kaib 1974; Kalinova et al. 2009; Stensmyr et al. 2002). Insects are feasible organisms for olfactory research because electrophysiological methods have been established for many species (Kalinova et al. 2009; Schutz et al. 1999; Stensmyr et al. 2002; Thakeow et al. 2008; Weissbecker et al. 2004), and the molecular basis of insect olfaction has been investigated for decades (Voigt and Riddiford 1981; Wicher et al. 2008). The order Diptera, in which most necrophilic insect species are classified, is relatively easy to breed at a high reproduction rate. Using chemo-ecological methods that combine trace analysis and electrophysiology (Weissbecker et al. 2004), these insects can be used to identify core compounds from the volatile pattern of a dead vertebrate. The existence of a common core of decompositional odors has already been suggested (Dekierirschier et al. 2009). Carrion-visiting Diptera can be divided into generalists, such as Calliphora vicina or Lucilia caesar, and specialists that occur in certain stages of decay, such as Hydrotaea aenescens or Hydrotaea mete- orica (Matuszewski et al. 2010b). Both types of insects might identify core compounds of vertebrate decay, which occur at any time or in certain stages of decay. In general, insect antennae serve as information filters that help in identifying volatiles for different applications. For instance, a post-mortem interval-correlated qualitative and quantitative dynamic volatile pattern could give an estimate of the time since death (Statheropoulos et al. 2007; Vass et al. 2008) or selected volatiles may improve cadaver dog training (Hoffman et al. 2009). Dimethyl sulfide, dimethyl disulfide, and dimethyl trisulfide are already known as electrophysiologically active for the blowflies C. vicina and L. caesar (Stensmyr et al. 2002) and the burying beetles Nicrophorus vespillo and Nicrophorus vespilloides (Kalinova et al. 2009). Such volatiles should be reliable for routine application and could be detected by low-cost and compact trace analysis devices such as semi-conductor gas sensor systems (Kohl 2003; Paczkowski et al. 2011). The tracing of victims of natural disasters, such as those buried in earthquakes, as well as the tracking of victims of homicides whose bodies have been buried (Killam 2004; Statheropoulos et al. 2007; Vass et al. 2008) may be improved by such methods (Statheropoulos et al. 2006). The industrial application of a detector for early vertebrate post-mortem VOCs could help in determining the degree of spoilage or estimation of the storage time of fish and meat (Lindinger et al. 1998; Nicolay 2006; Winquist et al. 1993).
Although the formation of biogenic amines is known to be correlated with microbial growth on vertebrate tissue, most of these compounds have a low volatility and, therefore, are not useful for a volatile-based quality estimation. Histamine, putrescine, cadaverine, tyramine, tryptamine, β-phenylethylamine, spermine, and spermidine are reported to be the most important biogenic amines (Onal 2007). Especially putrescine and cadaverine were regarded as important volatile products of vertebrate decay; however, all of the listed amines have a low volatility and, therefore, are not frequently identified in analytical literature on cadaveric volatiles (Table 3). Together with lipid acid formation during the formation of adipocere, biogenic amines could be important in estimating the pmi by means of liquid chromatography or SPME liquid extraction (Onal 2007). However, gas sensor systems would most likely not be able to detect them. On the other hand, ammonia might play an important role in aging vertebrate tissue. Olafsdottir and Kristbersson (2006) discuss the performance of electronic noses for the quality estimation of fresh fish. As gas sensors are restricted in selecting single compounds from an odor, the authors refer to compound groups such as sulfur compounds, volatile amines like ammonia, esters, aldehydes, or alcohols. The quantitative emission of compounds from these groups did correlate with the signals of three gas sensors. Hence, fish storage time was

| Number | Publication                | Tissue                                    | Location                     | Sampling technique                                                                 |
|--------|----------------------------|-------------------------------------------|------------------------------|-----------------------------------------------------------------------------------|
| 1      | Dekeirsschieter et al. 2009 | Domestic pig                             | Belgium, forest              | Radiello, passive, 1 week, GC/MS                                                   |
| 2      | Dekeirsschieter et al. 2009 | Domestic pig                             | Belgium, rural               | Radiello, passive, 1 week, GC/MS                                                   |
| 3      | Dekeirsschieter et al. 2009 | Domestic pig                             | Belgium, urban               | Radiello, passive, 1 week, GC/MS                                                   |
| 4      | Vass et al. 2004           | Human                                     | USA, Tennessee, open woodland| Triple sorbent traps (Carbotrap, Carbotrap C, Carbosieve S-III), GC/MS             |
| 5      | Vass et al. 2008           | Human, bones of human, deer, dog          | USA, Tennessee, open woodland| Triple sorbent traps (Carbotrap, Carbotrap C, Carbosieve S-III), GC/MS             |
| 6      | Statheropoulos et al. 2005 | Human                                     | Greece, island of Samos      | Three-layer sampling sorbent glass tube (Carbograph 1, Carbograph 2, Carbosieve S-III), 5l, GC/MS |
| 7      | Statheropoulos et al. 2007 | Human                                     | Greece, eastern Attica       | Three-layer sampling sorbent glass tube (Carbopack B, Carbopack C, Carbosieve S-III), 5l, GC/MS |
| 8      | Hoffman et al. 2009        | Different human tissues                   | —                            | SPME (PDMS/DVB), for 20 and 40 min in glas vial, GC/MS                             |
| 9      | Haze et al. 2001           | Living human skin                        | Japan                        | TENAX-TA column (GL Science), 10l, GC/MS                                           |
| 10     | Yan et al. 2001            | Adipocere                                 | —                            | —                                                                                  |
| 11     | Forbes et al. 2005a        | Domestic pig adipocere                   | Australia, laboratory        | 1 μl of chloroform extract, HMDS esterification, GC/MS                            |
| 12     | Forbes et al. 2005b        | Domestic pig adipocere                   | Australia, laboratory        | 1 μl of chloroform extract, HMDS esterification, GC/MS                            |
| 13     | Forbes et al. 2005c        | Domestic pig adipocere                   | Australia, laboratory        | 1 μl of chloroform extract, HMDS esterification, GC/MS                            |
| 14     | Forbes et al. 2002         | Soil sample beneath coffin                | Australia                    | 1 μl of chloroform extract, HMDS esterification, GC/MS                            |
| 15     | Notter et al. 2009         | Domestic pig adipocere                   | Australia, laboratory        | 1 μl of derivatised sample, GC/MS                                                 |
| 16     | Nushida et al. 2008        | Human adipocere                           | Japan                        | Processed and derivatised sample, GC/MS                                            |
| 17     | Stutz et al. 1991          | Ground beef                               | Laboratory                   | Porapak trap, GC/MS                                                              |
| 18     | Ercolini et al. 2009       | Beef muscle, latissimus dorsi             | Laboratory                   | 5 g homogenized in 100 ml water and 30 g NaCl, SPME (CAR/DVB/PDMS) for 1 h, GC/MS |
| 19     | Zhang et al. 1992          | Ayu skin, arachidonic acid                | Japan                        | 30 ml (1 mg protein/ml) + 50 μM arachidonic acid; Tenax TA, 70 ml/min, 90 min, GC/MS |
| 20     | Kalinova et al. 2009       | Mouse (Mus musculus)                      | Laboratory                   | SPME (CAR/PDMS) for 15 min in glass container, GC×GC-TOFMS                        |
detectable for the sensor array in this experiment. Winquist et al. used an array of 15 gas sensors detecting hydrogen, hydrogen sulfide, amines, alcohols, saturated hydrocarbons, and humidity in order to estimate the spoilage of ground beef (Winquist et al. 1993). In this study, a three-layer-pattern recognition routine was used in order to train the electronic nose on the recognition of the type of meat and the storage time. It was possible to determine the type of meat and the storage time of ground pork and beef; however, the authors state that in this experiment variation was minimal in comparison to the potential variation in practice. The quantitative emissions of the compound classes may vary to a high extent and important volatile cues might be under the detection threshold for gas sensors. For instance, Lindinger et al. detected methanethiol in parts-per-billion concentrations in the headspace of beef meat stored at 22 °C (Lindinger et al. 1998). As already described in this review, methanethiol is a good candidate for meat spoilage detection; however, it has to be evaluated if gas sensors are able to detect the compound at such a concentration level. Furthermore, meat stored at chill temperatures will develop a psychrotrophic micro-fauna, consisting of lactic acid bacteria and Pseudomonas spp. or Enterobacteriaceae. The volatile profile of these bacteria will differ from the volatile profile of micro-organisms growing at ambient temperatures. Ercolini et al. investigated the odor profile of psychrotrophic micro-organisms and did not find methanethiol (Ercolini et al. 2009), which indicates that this compound might form only at elevated temperatures. Rajamaki et al. found an increasing quantity of dimethyl disulfide in the headspace of broiler chicken at an elevated temperature, which might have formed from methanethiol (Rajamaki et al. 2006). Hence, methanethiol or the associated methylated sulfides could be markers for the inappropriate storage of meat at elevated temperatures. In the study of Rajamäki et al., a commercial electronic nose with a principal component analysis detected hydrogen sulfide concentrations, which allowed one to determine spoilage on meat packed under a modified atmosphere (Rajamaki et al. 2006). However, gas sensor technology has to be improved to enable the detection of low concentrations of single compounds for a reliable spoilage estimation. This improvement can be achieved by immobilizing proteins on sensor surfaces to utilize the strong odor detection abilities in nature (Hou et al. 2005; Khanna et al. 2006) or by varying the operating temperature for enhanced gas sensor selectivity (Sauerwald et al. 2007).

Conclusion

This review highlights the VOC emissions of dead vertebrates based on data from experimental trace analysis and their formation. There is a correlation between the information on biochemical processes and phenomenological results. For some volatiles, the pathways of their formation are entirely unknown or not known in detail. In particular, the detailed processes of fat degradation in prokaryotes are unknown. Based on existing information in the literature, it may be possible to hypothesize a core pattern of volatile emission during decay. After death, the volatiles produced by ROS might be the first to give rise to changes, and this event would be accompanied by the increasing growth of microbes that metabolize the fat on the skin and lead to decomposition by the tissues’ own enzymes. Once the immune system fails, the microbes that are already present inside the respiratory tract or in the gastrointestinal tract would contribute to the volatile pattern of decay. The metabolic products of particular intestinal microbes cause bloating under anaerobic conditions. This influences the microbial species composition and therefore gives rise to other volatile metabolic products. Breakage of the skin leads to the entry of oxygen, which again favors different microbes, yielding different volatiles. In an oxidative environment, secondary volatile products might form. During advanced dry decay, most of the carbohydrates and proteins are degraded. The volatiles produced by ROS and the microbial decomposition of bone components might dominate at this stage. Throughout this process, microbes might interact and thereby influence the pattern of the volatiles released. At present, information on vertebrate decay is incomplete. Therefore, the idea of a core pattern of volatiles of vertebrate decay remains a hypothesis. Further research on the application of post-mortem volatiles should integrate the knowledge of microbiologists and forensic scientists in order to perform experiments on the biochemical origin of these volatiles. The evolutionary preadaptation of insect olfaction to the perception of decompositional volatiles may help in developing volatile-based methods for forensic science, such as post-mortem time estimation or cadaver dog training.

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