Production, Transformation and Determination of Volatile Fatty Acids in Farm Animals and in the Environment

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Abstract. The paper is a critical discussion of the production, transportation and determination of volatile fatty acids in rumens of ruminants. The odor nuisance of the emission of VFAs, often accompanied by volatile nitrogen- and sulfur-organic compounds from animal farming is also discussed.

Keywords: Volatile fatty acids (VFA), ruminant stomachs, VFA formation and transformation.

1. Introduction

In view of continuous human activity, the level of environmental pollution is rapidly growing. Volatile organic compounds play a significant role among the pollutants, including short chain monocarboxylic acids (SCCAs) or volatile fatty acids (VFAs). The highest concentrations of VFAs can be observed wherever organic matter fermentation processes occur. These places are not only municipal waste landfills or sewage treatment plants but also a number of other sources. Fermentation processes accompany industrial food processing (including dairy products, food product preservation, alcohols) and are also produced by living organisms (mainly in ruminant stomachs).

The setting, occurrence and distribution of SCCAs are very diverse and the demand for their determination is quite wide.

2. Production and Transformation of VFAs

Organic acids are produced by anaerobic bacterial fermentation in the small intestine [1] and in the fore stomach of ruminants [2]. The production of these compounds depends on the functioning of the organism. 5-10% of the acid produced is used as energy necessary to maintain life processes. Both production and absorption of short chain carboxylic acids have a large effect on the growth of epithelial cells, blood flow, regular secretory function and the absorptive functions of the rumen and large intestine. In ruminants, short chain acids can be partially metabolized into ketone compounds and lactic acid or used as an energy source in the epithelial cells in the rumen. Acetate concentration in the blood, which can fluctuate around $100\,\mu mol/dm^3$, may rise to a $mmol/dm^3$ level as a result of alcohol consumption in humans [3], in a ruminant after a meal [4], while in horses, during lactation [5]. The end products of microbial fermentation of carbohydrates include: volatile fatty acids, mainly acetic, propionic and butyric; and gases, such as carbon dioxide and methane. The most important end products of carbohydrate breakdown in the rumen are volatile fatty acids (VFAs). These acids are important because: • they are the major source (70%) of energy for the ruminant; • the proportions in which they are produced determine fat and protein content of milk. The ratio of the various volatile fatty acids produced depends on the type of feed being digested. Volatile fatty acids are absorbed through the walls of the rumen and are then transported in the blood to the liver where they are

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converted to other sources of energy. The energy produced is used to perform various functions (i.e. milk production, maintenance of body systems, pregnancy, growth).

The rumen microbes produce three primary volatile fatty acids: acetic (CH_3COOH), propionic (C_2H_5COOH), and butyric (C_3H_7COOH). The primary VFA is acetic acid which is a two carbon VFA produced mainly by the digestion of fiber; it represents 55 to 70 percent of the total VFA formed. Large amount of propionate is formed in the process of grain fermentation. Propionate or propionic acid is a three carbon VFA produced by starch and sugar digestion bacteria. Propionate is converted to glucose by the liver. Glucose is used to synthesize milk lactose sparing amino acids from gluconeogenesis. The level of propionate varies from 15 to 30 percent of the total VFA production. The third main VFA is butyric acid which contributes to 5 to 15 percent of the VFA produced. Butyrate, produced to a lesser extent than acetate and propionate, is used as an energy source and for milk fat synthesis. Sometimes lactate is also formed, especially under acidic conditions in the rumen. VFAs are actually waste products from the rumen microbes, however, the cow absorbs them from the rumen and uses as major source of energy [6]. When evaluating VFA patterns, the ratio of acetate to propionate or the A:P ratio (60 percent acetate:25 percent propionate or 2.4:1) reflects the rumen fermentation pattern. Under optimal rumen fermentation conditions, the A:P ratio should be greater than 2.2 to 1. High levels of acetate can indicate a high fiber-low fermentable carbohydrate content. High levels of propionic acid can indicate reduced fiber digestion and acidosis. VFA analysis in the field has not been performed, but would be a useful tool to evaluate rumen fermentation and digestion. Exemplary processes are presented in Fig. 1.

Odour nuisance accompanies animal farming and can be a big problem if a farm is situated close to residential or shopping areas. The compounds responsible for unpleasant smell are mainly mercaptans, amines, and volatile organic acids. To control the smell monitoring methods are needed.
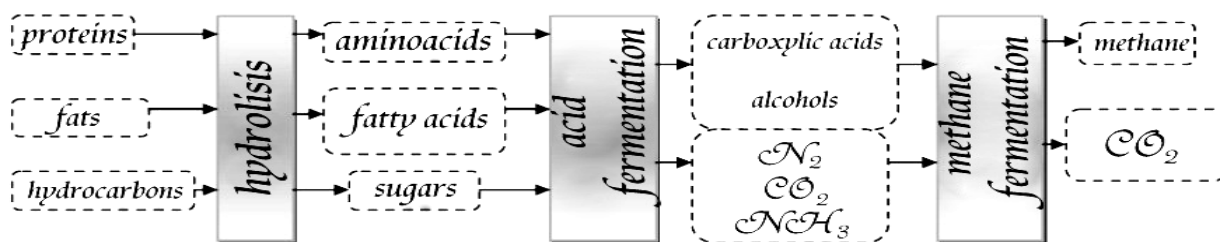

Fig.1: Exemplary processes proceeding in ruminants

3. Determination of VFAs

VFAs can be monitored either by determining the total quantity of acids in a sample or rather the content of each acid. In determination of individual organic acids in a variety of samples, separation techniques should be used such as: gas and liquid chromatography, including ion chromatography, and capillary electrophoresis. In most cases, samples must be specially prepared prior to injection to the measuring instruments.

Gas chromatography (GC) is usually a technique of choice because of quite a few reasons. Figure 2 shows the chromatogram of eight C2-C8 monocarboxylic acids. The mobile phase in GC is a neutral gas, which is environmentally friendly – it can be treated as a green mobile phase. Capillary columns, which are most often used consume rather small amounts of carrier gas and contain much less stationary phase and have a longer lifetime. Capillary GC has often been used as a technique of final determination of VFAs. The very significant progress has been achieved by introduction of the lab-on-a-chip analytical instruments which are faster and more flexible. A miniature device consists primarily of an injector and columns, and can separate mixtures of organic compounds within 10 seconds [7]. Now, miniature GC-MS systems, very powerful analytical tools are available. Their vacuum requirements are not very rigorous and carrier gas consumption is drastically reduced [8]. This is an inevitable trend that should lead to a breakthrough comparable to that brought about by computer introduction.

Liquid chromatography (LC) is superior to GC in that it can be used to determine volatile and non-volatile acids simultaneously. Increasingly often the reversed-phase LC columns (e.g. RP-C18 [9]) are used. When combined with UV-Vis detection at $\lambda = 215$ nm, LODs for SCCAs from 0.0023 to ca 10 mg/dm^3 can be achieved. Stavova et al. [10], for example, applied LC coupled with a high-resolution mass spectrometer (MS) or with tandem MS/MS to determine monocarboxylic acids (C1-C18) and dicarboxylic acids (C2-C14). Ultra-high performance liquid chromatography (UHPLC) increasingly often used to determine carboxylic acids [11] has pro-environmental nature. LC can be made greener using less harmful solvents as mobile phases under some special conditions. Ethanol, e.g., in typical conditions has poorer elution properties than the solvents generally applied but its elution capability can be improved, to a high degree by raising the temperature of separation [12]. In progress are the studies on the application of water as a LC mobile phase in the subcritical state. Under normal conditions, the solubility of NaCl in water is ca. 37%; while it is only ca 100 mg/dm^3 in subcritical water. The opposite trend is observed in the case of non-polar and slightly polar organic compounds which are poorly soluble in liquid water but well soluble in subcritical water [13]. Nonpolar carbon dioxide dissolves many organic compounds under supercritical conditions and it is often used as mobile phase in chromatography. Supercritical chromatography is increasingly often used to separate organics. When polar modifiers are added to CO_2 then polar organic compounds can also be separated.

Carboxylic acids can dissociate, and if the pH is sufficiently high, they occur mainly in ionized form and can be separated as carboxylic anions in anion-exchange chromatography (AEC). At low pH they can be separated using ion exclusion chromatography (IEC). An advantage of ion chromatography is that aqueous samples do not normally require any initial preparation other than filtration prior to the injection of the liquid sample into the chromatograph. To protect analytical columns from degradation and to prolong their lifetime, gourd columns are used. The detection is generally based on conductivity - suppressors are used to decrease conductivity of the mobile phase. Of the 21 applications of AEC for determining organic acids described by Peldszus [14], methanol and acetonitrile were used only twice. For determining VFAs the mobile phase can be aqueous solution of KOH [15]. The principal shortcoming of AEC is its poor resolution compared with typical LC. The retention mechanisms in IEC are very complex (Donnan exclusion, adsorption, polar interactions, hydrogen bonds etc.) [16].

Capillary electrophoresis is a technique requiring very small sample volumes, frequently nL and even pL. Because of low consumption of regents and solvents and the small sample volume, CE can be regarded as the greenest among the separation techniques. The detection system normally used in CE is the UV-Vis spectrometer, but this is not particularly sensitive, and when samples are small, LODs are relatively high. Carboxylic acids have a low coefficient of absorption, and often indirect analytical techniques must be used. In such a case LODs on the level of 2.0×10^{-6} mol/dm^3 were obtained for the following acids: oxalic, citric, tartaric, malic, succinic, acetic, lactic, aspartic, glutamic, ascorbic and gluconic [13]. CE with UV detection was used for the indirect determination of nine organic anions (formate, acetate, propionate, butyrate, oxalate, malonate, succinate, phthalate and maleate) and seven inorganic anions [17]. LODs below 0.5 mg/dm^3 were achieved for all these analytes except phthalate, for which the LOD was 1.0mg/dm^3. This confirms the applicability of CE for the determination of saturated and unsaturated mono- and dicarboxylic acids [17]. The LODs can be lowered by converting analytes into derivatives for which the detector is more sensitive and selective, especially if acids are converted to derivatives that fluoresce. The organic acids that can be occur in such beverages as wine, beer and fruit/vegetable juices were well separated and identified using CE and a photodiode detector following their derivatization into hydrazides. Electrophoretic analysis lasted 12 minutes and LODs were from 2 to 10.0 mg/dm^3. CE can be made environmentally friendlier if it is miniaturized. Such versions of CE are highly integrated, consume only small amounts of solvents and reagents, are highly efficient and quick. They have applied in conjunction with electrochemical detection to determine many environmental pollutants, including certain organic acids.
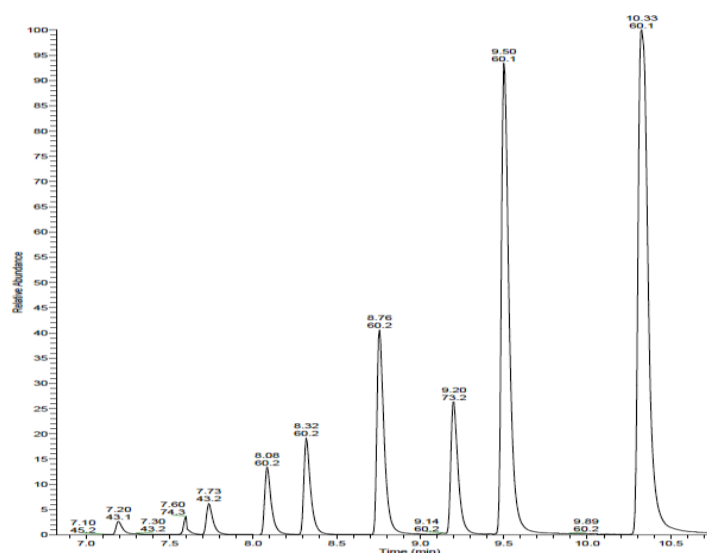
Fig. 2: GC chromatogram of VFAs standard mixture in water of eight acids: acetic (7.20), propionic (7.60), isobutyric (7.73), butyric (8.08), isovaleric (8.32), IS (8.76), valeric (9.20), caproic (9.50) and octane (10.33) acids on stabilwax capillary column (30 m, 0.25 mm I.D., 0.25 μm film thickness, Restek, Poland).

4. Conclusion

Animal farming is responsible for odour nuisance. It is strongly dependent on kind of animals, the way of their feeding and keeping, building for livestock, open containers for liquid manure, manure plates, etc., distance of farms from residential areas, shopping centres, etc. Malodorous compounds include VFAs and their emission must be controlled and monitored. VFAs differ in nuisance and individual acids should be determined. Separation methods such as chromatography and electrophoresis are generally used for acids monitoring and determination. The chromatographic techniques differ strongly in sensitivity. Often gas chromatography is a method of choice in the case of VFAs, especially if low concentrations are to be monitored. For simultaneous determination of volatile and non-volatile acids liquid chromatographic techniques and electrophoresis should be used. However, very often analyte acids must be pre-concentrated before the analysis proper.

The studies are being conducted to develop new analytical procedures. A special stress put on pro-ecological procedures and great progress is observed in the field.

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