Photoacoustic Monitoring of the Time Course of Ammonia Formation During the Spoilage of Inoculated Beef at Room Temperature

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1 Introduction

The natural aerobic spoilage of meat and that of meat inoculated with pure cultures is a complex process caused by microbial growth associated with the onset of various energy metabolisms. Chemical changes characterizing the end phase of bacterial activity at each of the distinct stages of their growth lead to the production of a well-defined sequence of volatiles [1]. In microbiology, determination of the microbial count number (number of bacteria per unit surface of meat) by elaborate plate counting techniques has become the common way to follow the growth and establish spoilage criteria. In principle predictions of the shelf-life of various meat products can be made if the relationship between the microbial count and the concentration of a specific volatile as the indicator of product deterioration can be found.

2 Experimental

A CO₂ waveguide laser in conjunction with a sensitive PA cell of the "organ-pipe" type [2], shown in Fig.1 was used in this study. There exist a considerable number of spectral coincidences between the CO₂ laser lines and the absorption lines for many of the identified volatiles, and for a number of them the absorption coefficients are also known. Slices of meat (3 cm x 3 cm x 0.5 cm) were sterilized (10 kGray γ radiation) and then inoculated (initial count 10⁴ bacteria/cm²) with a pure culture of either Pseudomonas putida or Brochotrix thermosphacta strain, bacteria known to dominate the spoilage flora at chill temperatures. Beef, placed in a 100 ml Pyrex flask, was flushed by the environmental air at a constant flow rate of 1.5 cm³/sec carrying away the volatiles from the head space to the PA

Fig.1. "Organ-pipe" resonant PA cell used in this experiment [2].
cell, thereby passing through the Zeolite molecular sieve and/or KOH traps to reduce water vapour and carbon dioxide content. A three-way valve enabled quick connection to the inoculated control sample whenever required. The measurements were carried out (at 18°C and pressure in the PA cell slightly below the atmospheric value) automatically using a HP 3421 acquisition data unit that samples vector output signal and the input laser power at selected wavelengths.

3 Results

The initial experiment had two goals: i) to find out whether the CO₂ laser can provide specific detection of some species produced in the early stage of spoilage and ii) to study the formation of ammonia known to take place at later time. Acetoin, an aliphatic monoketone is reported to be a compound produced in the earliest phase [1].

![Fig.2. Absorption coefficient of acetoin (in 10 μ band) at 291 K and 1 atmospheric pressure.](image)

The plot of its measured absorption coefficient versus the laser emission in 10 μm region shown in Fig.2 has a "grasslike" appearance. The maximum absorption in the 10R band is about 40 times lower than for example, that of ethyl acetate (another constituent found during the initial phase) in 9P band. The time course of beef spoilage was studied at discrete but regular intervals over a period of several days. The presence of acetoin (at sub-ppmv level) was found in the head space of both samples. A strong ammonia concentration gradient was observed between 30 and 60 hours after the start of the experiment, with the NH₃ concentration reaching a level of several ppmv, which is probably due to glucose depletion and the onset of aminoacid metabolism. Based on data collected in a parallel experiment that related the microbial count to the spoilage duration, bacterial densities of the order of 10⁶-10¹⁰ /cm² are expected within the same time interval. Our results suggest that the growth of Pseudomonas exceeded that of Brochothrix under the given experimental conditions. Towards the end of the third day, a decrease of NH₃ concentration was experienced (this finding is confirmed to a satisfactory extent by NEN 6472 (ISO/DIS 7150), a standardized colorimetric test for ammonia) indicating the growth reduction as shown in Fig.3. However, the