Microbial population analyses by FT-IR microspectroscopy

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Red smear cheeses such as Limburger, Romadur, Tilsit or Appenzeller are surface-ripened cheeses and are characterised by their complex microbial ripening flora which plays a major role in colour and aroma development. Floras of red smear cheeses mainly comprise coryneform bacteria, micrococci and yeasts in an unknown composition. To assure a consistent product quality and to prevent ripening failures like abnormal colour development due to progression of undesired bacteria or moulds, a monitoring of the consortium is useful. However, gaining a detailed look into the composition of a flora needs isolation and identification of large numbers of organisms and is therefore very laborious and cost-intensive.

FT-IR microspectroscopy may be a rapid alternative to conventional identification techniques as it provides a high degree of automation by combining a microscope with an FT-IR spectrometer. This enables the measurement of microcolonies and has already been shown to be a valuable tool for the rapid identification of yeasts [1]. Microcolonies obtained by dilution plating of samples are transferred from the agar plate to an infrared-transparent sample carrier by replica stamping and are measured in series enabled by a computer-driven *xy*-stage. Per day, approx. 200 colonies can be measured and identified, which allows direct analysis of populations without preliminary isolation of organisms and greatly shortens the time needed for an analysis. In the presented work, FT-IR microspectroscopy was applied to the population analysis of the bacterial surface microflora of a Vorarlberger Bergkäse and an Appenzeller cheese.

At first, a reference library comprising average spectra from 65 strains of 18 different coryneform species and staphylococci was elaborated. For identification the parameters proposed by Oberreuter et al. [2] were found to yield best results. For the population analyses 3170 spectra were recorded and identified altogether. To check the obtained identification results selected organisms were isolated and identified applying conventional FT-IR spectroscopy and partial 16S rDNA sequencing. Species well represented in the database could be identified reliably. Three species that could not be identified either belonged to a different taxon (e.g. lactic acid bacteria) or were novel species not included in the database. The obtained results demonstrate that FT-IR microspectroscopy is well suited for monitoring ripening consortia without applying molecular techniques and with reasonable work effort for routine analyses.

1. **Wenning, M., H. Seiler and S. Scherer.** 2002. Fourier-Transform Infrared Microspectroscopy, a Novel and Rapid Tool for Identification of Yeasts. Appl. Environ. Micobiol. **68**:4717-21.
2. **Oberreuter, H., H. Seiler and S. Scherer.** 2002. Identification of coryneform bacteria and related taxa by Fourier-transform infrared (FT-IR) spectroscopy. Int. J. Syst. Evol. Microbiol. **52:**91-100.