## EFFECT OF STARTER CULTURE ON FLAVOUR DEVELOPMENT OF FERMENTED SAUSAGES

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**Background.**

GC-MS is a powerful method for the analysis of flavour profiles. Complex flavour profiles can be analysed in great detail with respect to the identity of the compounds present and the intensity ratios between the compounds. However, visual inspection only of complex profiles is usually insufficient in detecting changes in compound ratios and correlations between (groups of) compounds. In these cases, multivariate data analysis (MVA) is mandatory to detect relations between compounds and to unravel biochemical pathways of formation. Moreover, MVA enables the selective identification of peaks with relevance to the trends and differences observed.

In the experiments described, batters were inoculated with bacterial strains (*Lactobacillus* FC1, *Staphylococcus carnosus*) and their respective enzymic extracts. Batters contained rifampicin to repress growth of the endogenous background flora. Volatile compounds from batters were extracted and analysed by GC-MS before inoculation and at three different time points during fermentation. Principal components analysis (PCA) was applied to the total ion chromatograms (TIC), resulting in scores and loadings plots. Differences in flavour formation during ripening caused by the strains and enzymic mixtures applied were monitored by these plots.

**Objective.**

To investigate the effect of bacterial starter cultures and enzymic extracts on the formation of flavour compounds in fermented meat products.

**Methods.**

*Experimental setup, sampling and storage.* Batters were inoculated with the mixtures in Table 1. Both the *Lactobacillus* and the *Staphylococcus* strain were made rifampicin resistant by serial transfer in media with increasing concentrations of rifampicin (until 200 microgram/ml). Batters were vacuum packed and incubated for 3 days at 25 °C. Ripening was performed at 12 °C. Samples were taken at t=0; 24 and 66 hrs; and 3 weeks and stored at -80 °C until analysis.

*Likens-Nickerson extraction, GC-MS analysis, MVA and data preprocessing.* Extractions were carried out by steam distillation-extraction with pentane-ether 1:1 (V/V). After extraction, the extracts were carefully concentrated. GC-MS analysis was carried out using a HP MSD 5973, G1530A GC-MS system. Spectra were obtained by full scan analysis. Data analysis of the GC-MS TIC profiles was carried out using MATLAB. Prior to MVA, a shift routine was applied to the TIC chromatograms of the entire data set in order to correct for small variations in retention time. Subsequently, PCA was applied using all time points (spectra) of the chromatograms. Scores and loadings plots were produced. The scores plots represent the shifts in position of the chromatograms during fermentation. The loading plots visualize the changes in concentration of the individual compounds.The latter were calculated in the form of complete chromatograms which enables the visualization of peaks with relevance for the trends and differences observed in the score plots.

**Table 1. Experimental set-up; additions at t=0.**

| Experiment | Bacterial strain ($10^7$ cells) | Cell free extract (of $10^9$ cells) | Experiment | Bacterial strain ($10^7$ cells) | Cell free extract (of $10^9$ cells) |
|---|---|---|---|---|---|
| B | *Lactobacillus* FC1 | | F | *Lactobacillus* FC1, *Staphylococcus carnosus* | |
| C | | *Lactobacillus* FC1 | G | *Lactobacillus* FC1 | *Staphylococcus carnosus* |
| D | *Lactobacillus* FC1 | *Lactobacillus* FC1 | H | *Lactobacillus* FC1, *Staphylococcus carnosus* | *Staphylococcus carnosus* |
| E (control) | - | - | | | |

**Results and Discussion.**

*Visual analysis.* In Figures 1 and 2 examples of the TIC chromatograms are shown. In the chromatograms of the control experiment (E0-E7) a gradual increase in concentration of hexadecanal, and the higher fatty acids tetradecanoic acid, hexadecenoic acid, hexadecanoic acid, octadecadienoic acid, oleic acid and octadecanoic acid was observed (Fig. 1). In experiment H a strong increase in concentration during fermentation of the same group of higher fatty acids and hexadecanal occurred. However, a second major effect was visible: an increasing concentration of lower aldehydes and fatty acids in time. This effect was amongst others observed for peaks 4: pentanal; 9: hexanal; 17: heptanal; 32: decenal; 34: decadienal; 35: decadienal (isomer); and 36: decanoic acid (Table 2). These shorter chain oxidation products were absent in the control experiment (E). The identified compounds are represented in Table 2.

*Description of the trends in the scores and loadings plots.* In the scores plot (PC 1 *vs.* 2, Fig. 3) a survey is presented of the main trends ocurring during fermentation. The corresponding chromatographic loadings patterns of PC 1 and 2 are given in Fig. 4. The spectra of the control experiment showed a shift in a different direction compared to the inoculation experiments. During fermentation of the control apparently no lower chain aldehydes and acids were formed. The experiments with added bacteria generally showed an increase in the concentration of lower aldehydes and fatty acids. This tendency is mainly represented by the shift along PC 2 which is the dominant axis of short chain aldehyde and acid formation. According to the positions in the scores plot it can

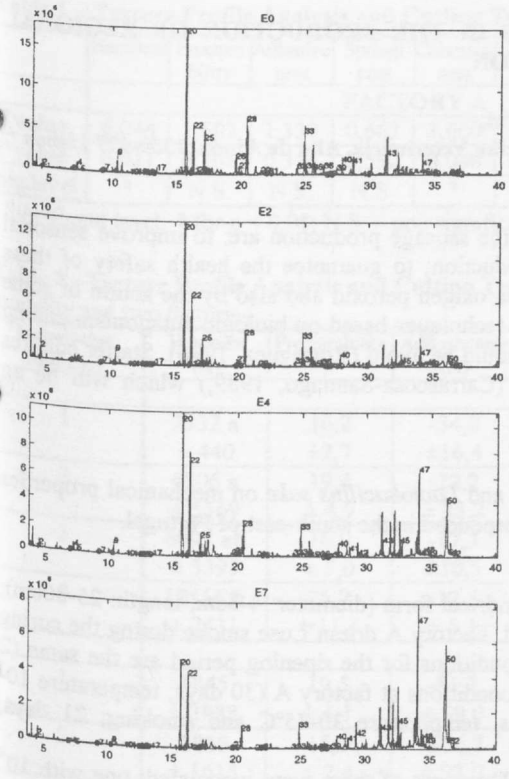


Figure 1. Control

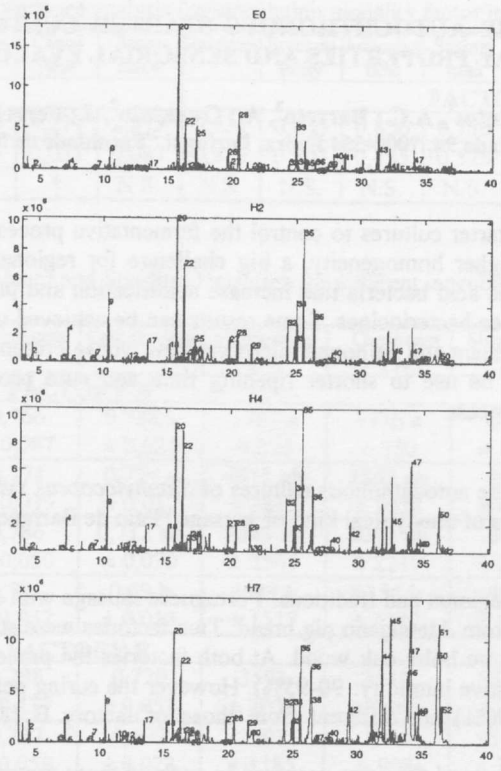


Figure 2. Experiment H

Table 2.

1. acetic acid
2. ethylacetate
3. 1-penten-3-ol
4. pentanal
5. 3-hydroxy-2-butanone
6. pentenal
7. 1-pentanol
8. butanoic acid
9. hexanal
10. furfural
12. 4-hydroxy-4-methyl-2-pentanone
13. hexenal
14. 3-methyl-butanoic acid
15. 2-methyl-butanoic acid
16. 1-hexanol
17. heptanal
18. heptenal
19. heptanol
20. a siloxane
21. 1-octen-3-ol
22. 2-pentylfuran + pentamethylheptane
23. octanal
24. heptadienal
27. nonanal
28. a siloxane
29. nonenal
30. octanoic acid
32. decenal
33. a siloxane
34. decadienal
35. decadienal
36. decanoic acid
37. decanoic acid, ethyl ester
38. undecenal
39. undecadienal
46. hexadecenoic acid
40. a siloxane
41. BHT
42. dodecanoic acid
43. unknown
44. tetradecanoic acid
45. hexadecanal
47. hexadecanoic acid
48. octadecenal
49. octadecanal
50. octadecadienoic acid
51. oleic acid
52. octadecanoic acid

be inferred that the chromatograms H2, G4 and H4 show relative high levels of short chain aldehydes and fatty acids. The shift to the left in the scores plot along PC 1 is mainly caused by the increase of long chain fatty acid and aldehyde concentrations. Especially for the chromatograms H7 and F7 the formation of high concentrations of long chain aldehydes and fatty acids during the last phase of the fermentation is reflected in the scores plot.

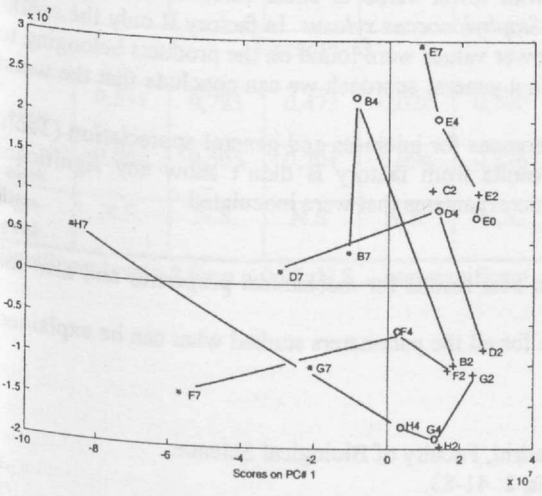


Figure 3. Score plot PC 1 *vs.* PC2.

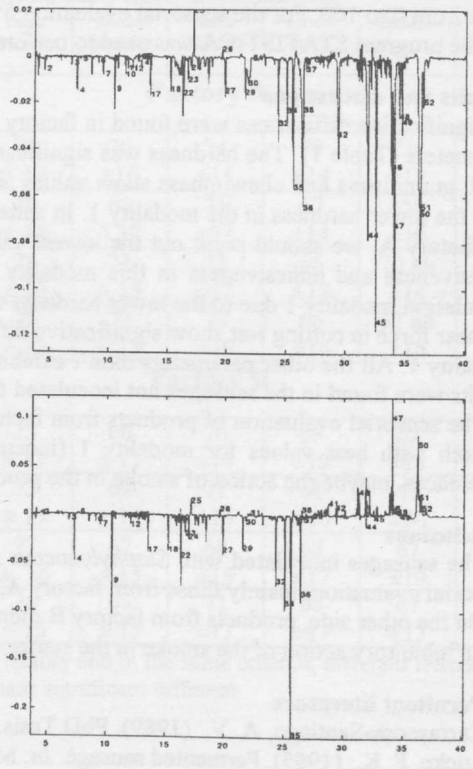

Figure 4. Loading patterns; PC1 (top) and PC2 (bottom).