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# PRODUCTION OF AROMA COMPONENTS BY *Staphylococcus* IN FERMENTED SAUSAGES

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## INTRODUCTION

The characteristic aroma of fermented sausage is due to a combination of many different components. Some originate from added spices, others are metabolic or chemical products created from carbohydrates, lipids and proteins during the fermentation and drying periods. Microbial growth in the sausage mince and activity of enzymes from the meat and fat are undoubtedly responsible for many of those compounds, however, autoxidative reactions may be of great importance as well. It is unknown which processes play the major part in the aroma development.

*Staphylococcus* is common to naturally fermented sausages (Nychas and Arkoudelos, 1990) and is believed to have a positive impact on flavour (Lücke, 1985). Commercial preparations of *S. xylosus* are therefore widely used in industrial and small-scale production plants. It is, however, unknown in what way *S. xylosus* gives rise to a better flavour.

The purpose of this study was to investigate the production of aroma components by *S. xylosus* in fermented sausages without spices. In order to have a well-defined control sausage without microbial growth a fungicide and two antibiotics were added to the control minces.

## MATERIALS AND METHODS

Sausages with and without *S. xylosus* were produced in four replicates. The control sausage minces without added *S. xylosus* were treated with chloramphenicol and tetracycline (100ppm) to inhibit bacterial growth, and potassium sorbate (0.2%) to inhibit yeast and fungi. The sausages were fermented and dried at 15°C for 28 days, analyzed for volatile content and evaluated sensorially.

Headspace volatiles from 50g of frozen ground sausage were equilibrated for 30 minutes at 37°C and purged with nitrogen (grade N50:>99.999% flow rate 150mL/min) through a Tenax TA (200mg) tube at 37°C for 30 min. Tubes were conditioned prior to sampling by a nitrogen flow of 15 mL/min for 2 hours at 300°C. Charcoal tubes (SKC inc, no.226-01, 50/100mg) were prepared in a similar manner.

Tenax tubes were thermally desorbed and injected into a gas chromatograph (Hewlett-Packard 5890 series II) by an automatic thermal desorber (ATD50 Perkin-Elmer). The GC was equipped with a 30m x 0.32mm id. DB-1701 fused silica capillary coloumn (J&W Sci.) connected to a flame ionization detector (FID). The oven was programmed from 30 to 225°C. Carrier gas (He) velocity was 30cm/sec (100°C), injector 250°C and detector 270°C. The desorption procedure was calibrated at every session by desorbing three calibration tubes containing 5µL of a 0.01% octane in methanol solution. The calibration tubes were prepared according to Perkin-Elmer: Thermal Desorption Data Sheet No.1.

Charcoal tubes were extracted with 1.5mL of diethylether (high purity grade, Merck no.929) into an injection vial and concentrated to 0.05 mL by gently blowing nitrogen over the ether surface. The concentrates were analyzed by GC/MS.

Hewlett-Packard Model 5890 GC interfaced to a TRIO 2000 (VG Biotech, Cheshire). Ionization energy 70eV, scan time 0.60 sec, transfer line temp. 250°C. The injections were splitless, purge time 0.70 minutes. Identification was based on Kovats retention index of authentic compounds and of MS spectra compared to the NBS/NIST-database.

The GC-results were analyzed by `principal component analysis' on peak areas using Sirius (version 2.2, Pattern Recognition Systems Ltd, Bergen, Norway).

The gas chromatographic effluent was evaluated by different members of the staff by sniffing at the outlet of the GC-column via an `olfactory detector outlet' (ODO-1, SGE). Split ratio 1:1. Their response to the odour from the outlet was timed and recorded by a second person. Six assesors were used for each type of sausage.
Sensory (only the odour) evaluation of the sausages was performed by a trained panel of 10 members using a quantitative descriptive method with 10 descriptors and a line scale of 15cm.

## RESULTS AND DISCUSSION

The main results of GC/MS are compiled in Table 1. Several alkanes and alkenes were identified, but they have been omitted from the statistical analysis as they most likely have no significant impact on flavour and because most of them could be contaminants from the wrapping material of the meat and fat used in the production. Thus, for many branched alkanes were shown to be present in the plastic wrapping. Also several aromatic compounds like benzene, methylbenzene, ethylmethylbenzene and trimethylbenzene were identified but were suspected of being contaminants as well. Several of the identified components have been detected by other workers (Cantoni *et al.*, 1967; Langner, 196;, 1972; Halvarson, 1973; Berger *et al.*, 1990, Berdagué *et al.*, 1992). This is especially true for the straight-chain alkanals and 2-ketones. However, except for ethyl acetate (Berdagué et al., 1992) none of the esters have been detected by others, even though many of the esters are also present in commercial sausages (Stahnke and Zeuthen, 1992) and are not characteristic for our types of sausages.

Figure 1 shows the score/loading plot from the `principal component analysis' on the peak areas of the identified compounds. Only the numbered compounds are included in the statistical analysis. The ones left out are either overlapping other peaks or present in too low a concentration to be quantified properly. The plot shows that sausages produced with *S. xylosus* are different from sausages added antibiotics. It also shows the compounds which are responsible for the separation of the two clusters. The greater variation between the individual sausages with *Staph.xylosus* compared to the variations between the control sausages is most likely caused by differences in microbial growth in those sausages.

The loading plot in Figure 2 demonstrates perhaps more clearly the compounds drawing the two clusters apart. Briefly, sausages with *S. xylosus* contain many esters, most of which are not present in the control sausages. Control sausages have higher concentrations of aldehydes and contain aldehydes that are not present in the sausages with *S. xylosus*.

The subjective odour responses obtained by effluent evaluation were numerous, many were not possible to assign to an identified compound. The results demonstrated, however, that many of the esters (no.17,815,20,25,29,30,32), even though their concentrations were low, and the aldehydes (e.g., no.11, 12, 16, 27, 36, 1087, 40) were easily detectable, while some of the major peaks (e.g., no.1, 2, 18, 24, 33) were non-odourous at the collected concentration level. The esters had fruity, candy and chutney-like notes, the aldehydes greenish, synthetic, deep-fried and herb-like smells. Considering that the sensory panel rated the sausages with antibiotics as being more rancid and unpleasant and the sausages with *S. xylosus* more like a typical, salty sausage, a preliminary conclusion is that the esters produced by *S. xylosus* play a role in the proper sausage aroma. It is also tempting to conclude that *S. xylosus* have an anti-oxidative effect as shown by the smaller concentrations of typical oxidation products like the alkanals (Frankel, 1983). One should keep in mind, though, the possible oxidative effect of the antibiotics added to the control sausages.

## CONCLUSION

The study demonstrates that *S. xylosus* produces several esters while growing in fermented sausages; esters that are not found in control sausages without microbial growth. Control sausages have an unpleasant odour compared to sausages with *S. xylosus*. This indicates that the esterase activity of *S. xylosus* and/or other microorganisms is essential in order to obtain the proper fermented sausage aroma.

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Table 1. GC/MS data for fermented sausages.

| Comp Num. | RT-index | Compound name | With Staph. xylosus | With anitbiotics | Means of identification |
|---|---|---|---|---|---|
| 1 | 546 | acetaldehyde ?? | + | + | - |
| 2 | 564 | ethanol | + | + | RT/MS |
| 3 | 624 | 2-propanol | + | + | RT/MS |
| 4 | 632 | 2-methyl propanol | + | + | RT/MS |
| | 634 | 2-methyl-2-propanol | + | + | RT/MS |
| 5 | 641 | 2-methyl furan | + | + | MS |
| 6 | 671 | butanol | + | + | RT/MS |
| 7 | 675 | ethyl acetate | + | + | RT/MS |
| 8 | 687 | 2,3-butandione | + | + | RT/MS |
| 9 | 711 | 2-butanol | + | + | RT/MS |
| | 715 | 2-methyl-3-buten-2-ol | + | + | RT/MS |
| 10 | 731 | isopropyl acetate | + | + | RT/MS |
| 11 | 735 | 3-methyl butanol | + + | + | RT/MS |
| 12 | 739 | 2-methyl butanol | + | + | RT/MS |
| | 740 | 2-ethyl furan | + | + | MS |
| | 741 | 2-methyl-1-propanol | | + | RT/MS |
| 13 | 751 | 2-t-butenol1 | + | + | RT/MS |
| 14 | 775 | 2-pentanone | + | + | RT/MS |
| 15 | 770 | ethyl propionate | | | RT/MS |
| 16 | 780 | pentanol | + | + | RT/MS |
| 17 | 780 | propyl acetate | + | | RT/MS |
| | 782 | 1-butanol | + | + | RT/MS |
| 18 | 790 | 1-penten-3-ol | | + | RT/MS |
| | 793 | 2,3-pentadione | + | + | RT/MS |
| | 805 | dimethyl disulfide | + | + | RT/MS |
| | 815 | ethyl isobutanoate | + | | RT/MS |
| 19 | 827 | 4-methyl-2-pentanone | + | + | RT/MS |
| 20 | 838 | isobutyl acetate | | | RT/MS |
| 21 | 838 | 2-methyl-2-t-butenol | + | + | RT/MS |
| 22 | 840 | 3-methyl-2-pentanol | + | | (RT)/MS |
| 23 | 847 | 3-methyl-1-butanol | + | | RT/MS |
| 24 | 856 | 3-hydroxy-2-butanone | + | + | MS |
| 25 | 859 | ethyl butanoate | | + | RT/MS |
| 26 | 863 | 2-t-pentenol | + | + | RT/MS |
| 27 | 884 | hexanal | + | + | RT/MS |
| | 887 | 4-methyl-3-penten-2-one | + | | RT/MS |
| | 890 | methyl ethyldisulfide | | + | MS |
| 28 | 893 | 2-c-penten-1-ol | + | + | RT/MS |
| 29 | 904 | ethyl-2-methylbutanoate | + | | RT/MS |
| 30 | 910 | ethyl-3-methylbutanoate | + | | RT/MS |
| 31 | 950 | 3-methylhexanol | + | + | MS |
| 32 | 960 | ethyl pentanoate | + | | RT/MS |
| 33 | 967 | 2,3-butandiol | + | + | RT/MS |
| 34 | 978 | 1-hexanol | + | | RT/MS |
| 35 | 982 | 2-heptanone | + | + | RT/MS |
| 36 | 988 | heptanal | | + | RT/MS |
| 37 | 1003 | cyclohexanone | + | + | RT/MS |
| 38 | 1079 | 1-octen-3-ol | + | + | RT/MS |
| | 1087 | octanol | + | + | RT/MS |
| | 1122 | dihydro-2(3H)-furanone | + | + | MS |
| | 1179 | 1-nitrohexan | + | + | RT/MS |
| 39 | 1184 | 2-nonanone | + | + | RT/MS |

Table 1(cont). GC/MS data for fermented sausages .

| | | | | | |
|---|---|---|---|---|---|
| 40 | 1189 | nonanol | + | + | RT/MS |
| | 1251 | 3,5-dimethyl-2-cyclohexen-1-one | + | + | MS |
| 41 | | ethyl octanoate | | | RT/MS |
| | 1260 | 2-t-nonenol | + | + | RT/MS |
| | 1267 | decanol | + | + | RT/MS |
| | 1288 | 2,4-t,t-decadienol | + | | RT/MS |
| | 1467 | ethyl decanoate | | + | RT/MS |
| | 1448 | | + | | RT/MS |

a) RT: Retention index compared to index of authentic compound.
MS: Mass spectra compared to NBS-library spectra.
(RT):Retention index compared to index of isomer.