DRIED SAUSAGES FERMENTED WITH *Staphylococcus xylosus* AT DIFFERENT TEMPERATURES AND WITH DIFFERENT INGREDIENT LEVELS

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SUMMARY

Sausages added *Staphylococcus xylosus* were fermented at different temperatures and added different levels of salt, glucose, nitrite, nitrate and *Pediococcus pentosaceus* in accordance with a six factor fractional design. The volatile components were collected from the sausages by dynamic headspace sampling and their content in the sausage headspace quantified and identified by gas chromatography and mass spectrometry. Also, the odour of the sausages was evaluated by a trained panel using a quantitative descriptive method. The sensory profile was correlated to the experimental design and the volatile compounds by partial least squares analysis.

The study showed that salami odour within certain borders was reduced by increased fermentation temperature, salt and nitrate and by addition of *Pediococcus pentosaceus*. Salami odour was correlated with ethyl esters and methyl ketones as well as with high numbers of *S. xylosus*. 2- and 3-methylbutanal seemed to be of importance as well. Sourish note was apparently caused by especially acetic acid and butanoic acid. Cheesy notes were correlated with the volatile acids 2-methylpropionic acid, butanoic acid and 3-methylbutanoic acid.

Introduction

The aroma of fermented sausage is of great importance to the sausage quality, but little is known about the components that comprise the aroma profile and the ones of more significance to flavour. Neither is much known about the influence of sausage ingredients and fermentation parameters on the final sausage aroma.

Several studies have shown that the volatile profile of a fermented sausage is very complex, including components from many classes of compounds (Berger et al., 1990; Berdagué et al., 1993; Johansson et al., 1993; Stahnke, 1994). The unique aroma property of most foods is not due to one, but usually to many compounds, blended in a specific ratio. There are two approaches to evaluate the components of more importance. One approach is to pinpoint the more potent aroma components in the volatile fraction by gas chromatography olfactometry (effluent sniffing) (Grosch, 1993; Acree et al., 1984). A second approach is to produce a descriptive sensory profile of the food in question and employ different multivariate statistical methods to study the correlation beween the volatile profile and the sensory data. In this way, it may be possible to get an idea of which component or class of components that are responsible for specific aroma notes (Chien and Peppard, 1993; Martens, 1982).

The purpose of this study was to investigate the effect of fermentation temperature and some basic sausage ingredients on the sensory profile of a fermented sausage added *Staphylococcus xylosus*.

Materials and Methods

The experimental design was set up as a six factor fractional design at two levels with resolution IV. Two centre points were included, giving a total of 18 different sausage batches. The design was computed by MODDE version 1.2 (UMETRI AB, Umeå, Sweden). The factors were fermentation temperature, glucose, salt, nitrite, nitrate and the starter culture *Pediococcus pentosaceus*.

All of the 18 different sausage batches were made of 80% lean pork meat, 17.5% pork back fat, 1 g/kg *Staphylococcus xylosus* - corresponding to $1x10^7$ cfu/g (freezedried preparation FloraCarn SX from Chr.Hansens's Lab. A/S, Denmark) and salt, nitrite, nitrate, glucose and *Pediococcus pentosaceus* (freezedried preparation FloraCarn P-1 from Chr.Hansen's Lab. A/S, Denmark) according to the experimental design. The low and high levels of the last ingredients were: salt: 1.5 and 3.5 %w/w; nitrite: 50 and 300 ppm; nitrate: 0 and 0.2 %w/w; *P.pentosaceus*: 0 and 4.2 x 10^7 cfu/g. To avoid interfering aroma components none of the sausages were added spices or smoked during drying. The minces were stuffed into 40 mm diameter casings (Fuji cellulose film SFK, Denmark) and fermented at either 15°C or 25°C for 48 hours and dried at 15°C for 26 days.

Headspace volatiles from 50 g of frozen ground sausage were collected by a dynamic headspace sampling method. The ground sausage was weighed into a glass flask, equilibrated for 30 min at 37°C and purged with nitrogen (grade N_2>99.999%, flow rate 150 mL/min) through a Tenax TA^R tube (200 mg, 60/80 mesh, Buchem bv, Holland) at 37°C for 30 min. Triplicate tubes were made. Charcoal tubes (50/100 mg, SKC inc, USA) were prepared in a similar manner, but purged with 27 L of nitrogen. Tenax TA^R tubes were thermally desorbed by an automatic thermal desorber (ATD50, Perkin-Elmer Ltd) and charcoal tubes extracted with ether. Please refer to Stahnke (1994) for details of chromatography, chemical and bacterial analyses.

The odour of the sausages was evaluated twice by a trained panel of 11 members using a quantitative descriptive method with 10 descriptors and an unstructured line scale of 150 mm. The list of descriptors was developed during training sessions by the panel members. The panel was trained once a week for a total of 3 months prior to the testing sessions.

The relationship between the gas chromatographic analyses and the sensory data were evaluated by 'partial least squares analysis' (PLS) using a multivariate analysis program (Sirius, version 2.3, Pattern Recognition systems A/S, Bergen, Norway). The effects of the different factors on the sensory scores were evaluated and tested using multiple linear regression and analysis of variance (MODDE version 1.2, UMETRI AB, Umeå, Sweden).

Results and Discussion

Table 1 shows the results from the multiple linear regression between the sensory scores and the six factors. The table comprises the regression coefficients of the factors that have a significant influence on the strength of the different aroma notes. The size of the individual regression coefficients is proportional to the importance of the factor in question.

It appears that none of the factors affect the 'intensity' of the sausages. At the sensory sessions the 'intensity' was described as the overall sensation experienced at first after opening the glass jar with the sausage sample. The descriptor is probably too coarse to be of value in profiling the aroma of this type of sausages.

'Salami' aroma was reduced by temperature, amount of *P. pentosaceus*, nitrate and salt. However, one should keep in mind that those effects are valid only in the temperature and ingredient span of the experimental design (see materials and methods). I.e. by increasing the fermentation temperature from 15 to 25 °C and/or the salt concentration from 1.5 to 3.5 % the 'salami' aroma of the final sausage will decrease. It seems possible that salt and also *P. pentosaceus* inhibit growth of desirable aroma producing microorganisms in the sausage mince while the effects of temperature and nitrate are more difficult to explain.

Figure 1 displays a loading plot from the PLS analysis of the volatile components and the sensory data, table 2 shows the variance explained by the principal components (PC). The first PC explains 31 % of the variation in the sensory data set. This means that about one third of the sensory profile in the direction of PC 1 is accounted for by the volatile components. It is seen in figure 1 that in particular 'nauseous', 'rancid' and 'cheese' and their correlations with the aldehydes decanal, pentanal and hexanal, and the acids methylbutanoic, 2-methylpropionic and butanoic acid are responsible for this.

'Salami', 'sourdough' and 'sourish' are opposed to 'nauseous', 'rancid' and 'cheese', pulled towards the right by the 2-alkanones and the number of survived *S. xylosus* and towards the bottom of the plot by the ethyl esters. Also an earlier study showed that ethyl esters are important to salami aroma (Stahnke, 1994). A PLS plot of the principal components PC 3 and 4 (not shown in this paper) shows that the aldehydes 2- and 3-methylbutanal may be of importance to salami aroma as well.

Apparently the 'sourish' and 'sourdough' notes were caused by the same components as 'salami'. However, the PLS plot of PC 3 and 4 (not shown) showed that 'sourish' is also influenced by acetic and butanoic acid and perhaps by 2-heptanone and 2-nonanone. 'Sourdough' seems to be correlated with the cheesy smelling 2-methylpropionic, butanoic and methylbutanoic acids as well.

Conclusions

The study showed that salami odour was reduced by increased fermentation temperature, salt and nitrate and by addition of *P. pentosaceus*. Salami odour was correlated with ethyl esters and methyl ketones as well as with high numbers of *S. xylosus*. 2- and 3-methylbutanal seemed to be of importance as well. The 'sourish' note to the salami aroma was apparently caused by especially acetic and butanoic acid. 'Cheesy' notes were correlated with the volatile acids 2-methylpropionic, butanoic and 3-methylbutanoic acids.

The results indicate that sausages fermented in an old-fashioned way with nitrate and at low temperature are likely to possess a more intense salami odour than sausages fermented at high temperature and added nitrite, glucose and *P. pentosaceus*.

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Table 1 Significant main effects.
*** = $p<0.001$, **=$p<0.01$, *=$p<0.05$. TEM=fermentation temperature, PED=*P. pentosaceus*, NAT=nitrate, NIT=nitrite, GLU=glucose, SAL=salt. + or ÷ in front of a coefficient indicates whether the factor increases or decreases the intensity of the aroma note.

Table 2 PLS analysis of relative GC peak areas and sensory scores.

Figure 1 Loading plot from PLS analysis of sensory scores and content of volatile compounds. Table 2 shows the variation explained by the principal components.