VOLATILES PRODUCED BY *Staphylococcus xylosus* GROWING IN SAUSAGE MINCES

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Background

Several studies have shown that the volatile profile of fermented sausage is very complex, including more than hundred different compounds from many classes of components (e.g. 1,2). Microbial growth in the sausage mince together with the activity of enzymes from the meat and fat are responsible for many of those components. Also, autoxidative reactions are of great importance.

It is unknown which processes play the major part in the flavour development and how this flavour is related to microbial growth, ingredient levels and other production parameters. In one study correlation of sensory data and volatile, microbial profile indicated that salami aroma was related to the level of ethyl esters and certain short-chain, branched aldehydes, methyl ketones and the starter culture *Staphylococcus xylosus*. It was not possible though, to establish if this bacteria produced the compounds or if other microorganisms or mechanisms were involved (2).

Objectives

The purpose of the present study was to investigate the production of major volatile compounds by *Staphylococcus xylosus*. In order to avoid interference from background flora the bacteria was grown in aseptic model sausage minces. The experimental design was set up as a fractional factorial design, examining the influence of temperature, pH, oxygen level, concentration of salt, nitrite/nitrate, glucose and ascorbate. The factor levels were chosen to cover a wide spectrum of production parameters of different types of fermented sausages.

Methods

The experimental design was set up as a seven factor fractional design at two levels with resolution IV (2^{7-3} structure). Four centre points were included giving a total of 20 sausage minces. Main effects were confounded with three-factor interactions, two-factor interactions were confounded with each other.

Model minces were made of 45% w/w lean pork, 15% w/w pork back fat and 40% w/w water including ingredients according to the experimental design (cf. factorial levels in the footnote to table 1). All minces were added $5x10^7$ cfu/g *Staphylococcus xylosus* (Chr.Hansen's A/S, Denmark). The minces were placed in Erlenmeyer flasks mounted with two horizontal glass tubes closed with Swagelock fittings. At first, the minces were incubated for 4 days at the conditions specified in the experimental design. Secondly, all minces were equilibrated at 17°C without access to air for 18 hours. Finally, Tenax TA^R tubes (200 mg, 60/80 mesh) were fixed onto the glass tubes and headspace volatiles from the minces collected by passive diffusion for 5 days at 17°C. Duplicate tubes were made for each mince. The volatiles on the Tenax TA^R tubes were thermally desorbed (ATD50, Perkin-Elmer) and analyzed by GC/FID (flame ionization detection) and GC/MS (mass spectrometry) on Hewlett-Packard instrumentation. The separation was performed on a DB-1701 capillary coloumn (J&W Sci., USA). The desorption procedure was calibrated by desorbing 5 calibration tubes containing 5 μL of a 0.01% octane in methanol solution (3). Headspace volatiles were semi-quantified by dividing peak areas with the averaged area of the octane peaks from the 5 calibration tubes. Identification was based on Kovats retention indices of authentic compounds and of MS spectra compared to the NBS/NIST-database.

The results were evaluated and tested using multiple linear regression and analysis of variance (MODDE version 3.0, UMETRI AB, Umeå, Sweden). The GC peak areas were transformed into the logarithmic$_{10}$ scale prior to analysis.

Results and discussion

Table 1 shows the results from the regression analysis of the major headspace volatiles of the minces. Several other volatiles were identified but were not possible to quantify properly. All the identified components have been detected in earlier studies of fermented sausages (e.g. 1,2). Based on the statistical analysis the formation of some of the volatile components in relation to the experimental factors is discussed in the following.

The statistical analysis shows that the level of acetonitrile was increased by increasing amounts of nitrate and by raised temperature. The same relationship was shown between heptanonitrile, temperature and nitrate in fermented sausage in an earlier study (2). The occurence of nitriles in cured meat has also been reported by (4). It is uncertain whether the nitriles are formed directly by *S. xylosus* or by a chemical reaction. Several other nitriles were identified but not quantified.

The levels of 2-methylpropanal, 2-methyl- and 3-methylbutanal were increased by high temperature, low amounts of ascorbate and, most strongly, by low oxygen level during growth. The interaction effect, -TEM·ASC, shows that the temperature effect

was greatest at low ascorbate contents. These findings indicate that the aldehydes are produced by fermentative oxidation (-OX and -ASC) from the amino acids valine, isoleucine and leucine, respectively. Probably by oxidative deamination to the corresponding ∝-keto acid followed by decarboxylation. Normally, fermentation of amino acids produces acetic and butanoic acid and less amounts of propionic acid depending on the microorganism and amino acids involved (5). It is often suggested that 2-methylpropanal, 2-methyl- and 3-methylbutanal originate from non-enzymatic Strecker degradation (e.g. 1). This is not likely in this study since the water content of the minces was quite high (75-80% w/w).

The content of 2- and 3-methylbutanoic acid was increased by increasing amounts of nitrate, especially if the temperature was high (+TEM·NT). This indicates that the acids are produced from the corresponding aldehydes by chemical oxidation. Nitrate is an oxidant at acidic pH promoting e.g autoxidation of lipids (2). The levels of acetic, propionic and butanoic acid are also increased by high nitrate content and may be produced from the corresponding aldehydes as well. The influence of salt is puzzling, though. Ordinary amino acid fermentation may be involved (see above).

The levels of diacetyl and acetoin were increased by increasing amounts of glucose, showing that *S. xylosus* produces those compounds from glucose. Nitrate increased the amount of diacetyl, especially when temperature was high (+TEM·NAT), ascorbate decreased the amount (-ASC). This shows that high redox potential raises the content of diacetyl. Otherwise most of the diacetyl would probably convert into acetoin by acetoin dehydrogenase as is the case for lactic acid bacteria fermentation of diacetyl (5). This is also indicated by -NT·GLU, which shows that when no nitrate is present glucose is degraded all the way to acetoin (via diacetyl).

The levels of 2-propanone (acetone), 2-pentanone and 3-methyl-2-pentanone were all increased by access to oxygen, increasing salt concentration and decreasing glucose concentration. 2-alkanones may be formed by ß-oxidation of fatty acids or by decarboxylation of ß-keto acids which is perhaps the case. -GLU implies that *S. xylosus* only forms 2-alkanones when no easily degradable carbon and energy source is present (in this case glucose). The effects of salt and pH are puzzling.

The content of 1-penten-3-ol was increased by addition of nitrate, high salt concentration and low ascorbate content. This shows the oxidative effect of nitrate and the antioxidative effect of ascorbate since 1-pentene-3-ol is produced during lipid autoxidation. It seems as if salt works as an oxidant as well.

Conclusions

Staphylococcus xylosus produces a wide variety of volatile compounds when growing in fermented sausage mince. Many of those components have been shown to be of importance to fermented sausage flavour in an earlier study (2). The present study describes how the formation of major volatiles is influenced by production parameters when *S. xylosus* is growing as a pure culture. This has given a greater understanding on how *S. xylosus* affects the aroma profile of fermented sausage. Future work will continue the study of this subject.

Literature

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Table 1.

	Significant main effects[a]							Significant two-factor interactions[b]
	pH	TEM	SAL	NT	GLU	ASC	OX	
acetonitrile		+#		+***				
dimethyldisulfide		+*		−*				
2-methylpropanal		+*				−*	−***	−TEM·ASC*
3-methylbutanal		+*				−*	−***	−TEM·ASC#
2-methylbutanal		+*				−**	−***	−TEM·ASC*
3-methylbutanoic acid				+*				+TEM·NT*
2-methylbutanoic acid				+*				+TEM·NT*, +pH·TEM/ASC·OX/SAL·GLU*
acetic acid			+*	+***				−SAL·NT***, +SAL·ASC/TEM·NT**
propionic acid			+*	+***				
butanoic acid				+**				
ethyl acetate							+#	
3-methyl-1-butylacetate							+*	
acetone (2-propanone)			+***		−*		+***	−TEM·SAL/pH·GLU**
diacetyl (2,3-butadione) + 2-butanone		+***		+***	+***	−#		−pH·ASC***, +GLU·OX/TEM·NT*
acetoin (3-hydroxy-2-butanone)		+#			+*			−NT·GLU*
2-pentanone	+***	+#	+**		−***		+***	−GLU·OX/TEM·NT/SAL·ASC*, −PH·SAL/TEM·GLU/NT·OX*
3-methyl-2-pentanone	−*	+***	+***	−***	−**		+***	+pH·TEM/SAL·GLU/ASC·OX*, −TEM·OX/NT·GLU/pH·ASC*
2-propanol	+**		+#		−*		−*	
1-pentene-3-ol			+**	+*		−*		
3-methyl-1-butanol	+*				+#			−pH·OX*

[a] *** = p≤0.001, ** = p≤0.01, * = p≤0.05, # = p≤0.10. TEM=temperature (17-33°C), SAL=salt (2.4-9% w/w in water), NT=KNO_3:$NaNO_2$ (low level=150ppm NO_2, high level=0.2% NO_2), GLU=glucose (0-0.5 % w/w), ASC=ascorbate (0-500 ppm), OX=oxygen access (low level=flask closed, high level=flask closed with cotton ball). + (−) : compound level increases (decreases) by increasing the factorial level.

[b] Two-factor interactions may be confounded.