MICROBIAL FLAVOUR FORMATION IN A CURED PORK MODEL SYSTEM

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Background

The flavour of fermented meat products is a complex interaction of volatile compounds and water and fat soluble substances. However, recent investigations suggest that particularly amino acid derivatives are important for the overall flavour in Italian style dry-cured ham (Hinrichsen and Pedersen, 1995; Careri et al., 1993) and Wiltshire bacon (Andersen and Hinrichsen, 1995). In bacon it has been demonstrated that the natural occurring flora on the meat is able to produce these derivatives in a bacon model system (Hinrichsen and Andersen, 1994). Consequently, the improvement of flavour in other types of cured meats will be dependent on the ability of the resident microflora to produce amino acid derivatives.

Objectives

The aim of the present study was to select flavour improving starter cultures for cured meat products on the basis of the microorganisms' ability to produce volatile compounds, in particular derivatives of amino acids, in a cured meat model system.

Methods

The model system was made from sterile minced pork and bacon curing brine. Pork loin (24 h post mortem) was derinded, defatted and subsequently heat treated in boiling water for 120 sec. The surfaces were cut off and the sterile meat was minced under sterile conditions. Cells were suspended in sterile curing brine (3,6 M NaCl, 17 mM KNO_3 and 19 mM $NaNO_2$) and brine containing microorganisms was added the sterile meat to a weight increase of 16 %. The bacteria, all isolated from naturally fermented meat products, were 6 *Staphylococcus xylosus* (0072, M350, BS104, BS105, BS107 and BS108), 1 *Staphylococcus carnosus* (F833), 1 *Staphylococcus saprophyticus* (F831), 1 *Staphylococcus warneri* (F863), 1 *Staphylococcus* sp. (SF), 1 *Micrococcus roseus* (0012), 2 *Micrococcus* spp. (0025 and 0062), 1 *Lactobacillus* sp. (L45), 1 *Corynebacterium callunae* (0057), 1 *Halomonas elongata* (0013) and 3 *Vibrio* spp. (0091, 0104 and 0081). The bacteria strains were added at a level of 10^7 cfu/g with three replicates and the mixture of sterile minced meat, curing brine and microorganisms was incubated at 20°C for 7 days in Blue Cap flasks.
After incubation the number of bacteria were enumerated on plate count agar with 4% NaCl added, and counted after 5 days at 20°C. Volatile compounds were measured by head space gas chromatography-mass spectometry and determination of NO_3^-, NO_2^- and pH was performed as described by Hinrichsen and Pedersen (1995). The level of NO_3^-, NO_2^- and pH was evaluated by analysis of variance, whereas contents of volatile compounds were exposed to principal component analysis.

Results and discussion

Microbiological analysis showed that all *Staphylococcus* spp., *Micrococcus* spp. (0025 and 0062), *Lactobacillus* sp. (L45) and *Corynebacterium* sp. (0057) grew during incubation in the model system and cell numbers increased at least one log unit. The number of *Micrococcus* sp. (0012) and *Halomonas* sp. (0013) decreased one log unit under the same conditions. The cell number of the *Vibrio* spp. decreased drastically and strain number 0104 could not be detected.

Some of the added bacteria strains had an impact on pH ($P<0.001$). Some of them decreased the pH. However, *Staphylococcus xylosus* (M350), *Staphylococcus xylosus* (F831), *Staphylococcus warneri* (F863), *Staphylococcus xylosus* (BS104), *Micrococcus* sp. (0062) increased the pH. The increase of pH for these strains indicates catabolic activities on amino acids i.e. by deamination. In general all the strains were able to reduce nitrate ($P < 0.001$). In addition *Micrococcus* sp. (0062) also reduced nitrite ($P<0.001$). The concentration of nitrate and nitrite in samples inoculated with *Micrococcus* sp. (0012), *Vibrio* spp. (0091 and 0081) or *Lactobacillus* sp. (L45) remained unchanged compared to the control samples.

By principal component analysis (PCA) of volatile compounds in the meat samples after incubation it was possible to describe 77 % of the variation in the total data set (results not shown). PCA revealed that meat emulsions with added *Staphylococcus* spp. (SF or F831), *Micrococcus* sp. (0012), *Lactobacillus* sp. (L45), *Halomonas* sp. (0013) or *Vibrio* spp. (0091 or 0104) were similar to the uninoculated control samples. It seems therefore, that these strains do not produce any particular volatile compounds compared to the control samples. The strains *Staphylococcus xylosus* (0072), *Micrococcus* sp. (0025) and *Corynebacterium callunae* (0057) produce 3-methylbutanol, 3-methylbutylacetate, 4-methylpentan-2-one, 3- and 2-methylbutanal, ethylacetate, 3-methylbutanoic acid and diacetyl/butan-2-one. *Micrococcus* sp. (0062) produces butan-2-ol. *Staphylococcus xylosus* (M350) and *Staphylococcus warneri* (F863) produce 3-methylbutanol, 3- and 2-methylbutanal, ethylacetate, 2-methylpropanal, 3-methylbutylacetate and 3- and 2-methylbutanoic acid. *Staphylococcus carnosus* (F833) produces only 4-methylpentan-2-one and diacetyl/butan-2-one. *Staphylococcus xylosus* (BS104, BS105 and BS108) produces 3-methylbutanol, ethylacetate, 3-methylbutylacetate and 3-methylbutanoic acid. Finally, *Vibrio* sp. (0081) produces 4-methylpentan-2-one and diacetyl/butan-2-one. In table 1 the concentrations of volatiles with loadings larger than 0.2 are listed. Compounds characteristic for the different strains are marked in the table.

Table 1. Concentration in dodecane equivalent (ng) of most significant volatile compounds (n=3). Volatile compounds specific for the bacterial strains are shaded.

Compound		2-Me[1]-propanal	Ethyl-acetate	Diacetyl/ butan-2-on	Butan-2-ol	3-Me-butanal	2-Me-butanal	4-Me-pentan-2-on	3-Me-butanol	3-Me-butylacetate	3-Me-butanoic acid	2-Me-butanoic acid
Kovats' index		628	672	684	707	736	741	828	852	943	1020	1025
Staphylococcus xylosus	0072	81	301	5021	4	405	584	3211	46822	1023	133	83
Staphylococcus xylosus	M350	896	188	1098	8	3011	1912	560	27052	53	119	86
Staphylococcus sp.	SF	2	18	2103	9	9	4	1582	0	1	2	0
Staphylococcus carnosus	F833	342	496	2582	10	2139	1263	2096	474	213	381	242
Staphylococcus saprophyticus	F831	10	211	1135	7	36	31	464	5004	59	112	93
Staphylococcus warneri	F863	1024	182	1246	6	4510	1869	146	6543	85	156	85
Staphylococcus xylosus	BS104	5	455	2161	0	13	19	1420	3276	91	124	33
Staphylococcus xylosus	BS105	6	433	1211	0	19	23	1048	3296	115	302	182
Staphylococcus xylosus	BS107	10	336	747	0	19	15	693	972	32	228	212
Staphylococcus xylosus	BS108	35	393	746	0	110	231	1147	8960	303	300	179
Micrococcus roseus	0012	0	5	3848	2	0	4	1543	9	0	1	0
Micrococcus sp. grp. 1	0062	20	277	1380	370	280	116	613	1810	1	12	8
Micrococcus sp. grp. 2	0025	9	231	2758	0	16	13	2136	777	21	220	186
Lactobacillus sp.	L45	11	102	1418	6	2	8	2007	34	4	9	4
Corynebacterium callunae	0057	38	976	7388	2	190	131	12520	16535	451	192	159
Halomonas elongata	0013	4	5	3155	197	0	2	1134	8	0	0	0
Vibrio sp. grp. 1	0091	0	8	3139	1	3	4	930	11	0	2	0
Vibrio sp. grp. 3	0104	1	7	1835	9	1	2	1224	2	0	2	0
Vibrio sp. grp. 3	0081	3	9	4029	0	3	7	3794	9	0	4	0
Control		1	0	906	5	0	0	229	6	0	3	0

[1]Methyl

The results clearly demonstrate that even though the strains carry the same species name they have rather different abilities to produce volatile compounds. As the amino acid derived volatiles are particularly interesting for the flavour in cured meats, attention should be paid to the compounds 2-methylpropanal (derived from L-valin), 3- and 2-methylbutanal and 3-methylbutanol, 3-methylbutylacetate and 3- and 2-methylbutanoic acid (derived from L-leucine).

Thus, *Staphylococcus xylosus* (0072, M350 and BS108) and *Staphylococcus warneri* (F863) and *Corynebacterium callunae* (0057) produce high amounts of volatile compounds relevant for flavour in cured meat products. The action of these strains on amino acids seems not to affect pH as a general increase in pH is not observed. This indicates that the degradation mechanism does not involve deamination but rather transamination. The selected strains all reduce nitrate, but do apparently not reduce nitrite. These are all desirable abilities in meat products due to their colour stabilising effect.

Conclusions

Staphylococcus xylosus (0072, M350 and BS108) and *Staphylococcus warneri* (F863) and *Corynebacterium callunae* (0057) are able to produce relevant volatile compounds in a cured pork model system and may therefore serve as flavour improving starter cultures in cured meat products. However, application experiments in pilot scale will be necessary in order to verify the flavour improving abilities of these strains.

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Literature

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