AROMA PRODUCTION FROM MEAT STARTER CULTURES

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SUMMARY

Addition of starter cultures to fermented meat products contributes significantly to their flavor. In this work the aroma formation from meat starter cultures on pork fat was investigated by means of a model system with emulgated fat in order to study the production of flavor compounds of lipid origin. Volatiles were collected by a dynamic headspace sampling technique and analysed by GC-FID or GC-MS. Starter cultures investigated were *Staphylococcus xylosus, Staphylococcus carnosus, Lactobacillus plantarum, Lactobacillus alimentarius, Lactobacillus sake and Pediococcus pentosaceus.* The experiments have shown, that characteristic flavor compounds are produced in model systems with pork fat. Some of the flavor compounds have been identified and correspond well with metabolic intermediates derived from lipids. These include aldehydes, ketones, alcoholes, hydrocarbons, acids and esters. Major products are butan-1-ol, 2-methyl- and 3-methyl-butanals, 3-methyl-butan-1-ol, 2-methyl- and 3-methyl butanoic acid, hexanal as well as normal lactic acid bacterial fermentation products such as acetoin, 2,3-butanedione and 2,3-pentanedione. Furthermore, this investigation shows the differences between starter cultures in production of volatile compounds; each culture has its own characteristic "flavor profile".

Introduction.

Flavor formation in fermented meat products are a complicated process, involving enzymatic activity from both starter culture, natural flora and from enzymes in the meat itself. The enzymes includes lipases, proteases and probably esterases. Also autoxidation of lipids occur. In order to elucidate the effect of starter cultures on lipids in fermented meat products, we investigated the bacterial activity in a model system. The use of such a model system has the advantage of eliminating most of the volatile products resulting from proteolytic activity. Furthermore, processes in meat involving natural flora and meat enzymes are excluded.

The model system has previously been used to investigate the lipolytic activity by measuring the production of free fatty acids (Nielsen and Kemner, 1989; Kemner and Nielsen, 1990). We here present data for the use of the model system to investigate the production of volatile aroma compounds.

The volatile compounds were sampled by a dynamic headspace technique. The sampling temperature is critical (Ishihara and Honma, 1992). In order to avoid condensation of volatiles and water in cold zones, the whole sampling system was placed in an incubator. The temperature was held relatively low at 37 °C in order to minimize thermal formation of artifacts during purging.

The analysis of volatiles was done by thermal desorption of the Tenax tubes at 250 °C, followed by direct transfer of the desorped compounds to a GC column. The volatiles were detected using both FID and MS detectors.

Material and methods.

The model system: 10% pork fat emulgated with 18% gum arabic in a solution containing 0.35% yeast extract and 0.7% tryptone. The ingredients were mixed with an Ultra Turrax blender and the fat emulsion was autoclaved. pH was adjusted with 5 M phosphate buffer to a pH value of 8 prior to inoculation with starter culture.

Starter cultures: *Lactobacillus alimentarius, Lactobacillus plantarum, Lactobacillus sake, Pediococcus pentosaceus, Staphylococcus carnosus, Staphylococcus xylosus.* All starter cultures were from Chr. Hansen´s Laboratory. The bacteria were grown in CASO broth (Casein-Peptone Soymeal-peptone) at 25 °C to a dense

suspension. Fat emulsions were inoculated with 1,5 ml bacteria culture. Samples were incubated at 10 or 20°C for 10 days. All samples were made in duplicate. A blind reference was added 1,5 ml sterile water instead of bacteria culture. Two blind samples and two with *S.xylosus* were immediately frozen.

Sampling: A flow of purified nitrogen was purged through the sample flask, although not through the fat emulsion itself. Temperature was kept at 37°C. The volatiles were trapped on a Tenax TA 60-80 mesh filled metal tubes designed to fit a Perkin Elmer ATD-400 autosampler. The flow velocity was 100 ml/min for 30 minutes giving a total volume of 3 l for purging. The fat emulsion was stirred during the sampling period.

Desorption: temperature: 250 °C, flow: 22 ml/min, time 15 min, Split flow: 22 ml/min. Desorption was carried out on a Perkin-Elmer ATD-400 autosampler and directly transferred onto the CG column (J and W Sci. DB1701, 30 m, 0,25 mm id, 1μ).

Chromatographic systems: Perkin-Elmer Autosystem Gaschromatograf with FID detection. Hewlett Packard GC 5890 Serie II gaschromatograph with HP 5972 (quadropole) mass selective detector. Flow He (N 6,0) 1 ml/min. Temperature program: 35°C for 10 min, to 150°C with 3°C/min, hold for 5 min, to 250°C with 30°C/min, hold for 5 min. FID detector temperature 270°C. MS inter-face temperature 280°C (MS temperature 170°C). Scan 35-250 m/z with 2,2 scan/sec. Compounds were identified by comparison of retention times with known standards. Samples analysed by MS were identified by library search in NIST (NBS75K) library and by comparison of Kovat´s retention indices.

Results and discussion.

Conversion of lipids to various products contributes to the flavor of fermented meat products. The compounds produced are both volatile and non-volatile. Many contributes to a characteristic flavor of the meat product, while others give origin to off flavors.

Many of the starter cultures used for meat processing have been shown to exhibit lipolytic activity. The production of free fatty acids is a prelude to further breakdown, resulting in production of a wide range of volatile compounds. The model system consisted of pork fat emulsified with gum arabic. The emulsion was buffered with phosphate buffer in order to keep pH approximately 8 in spite of acid production from the starter culture. We have shown that optimum pH for lipolytic activity of these starter cultures was 8 (Nielsen and Kemner, 1989).

In order to produce considerable amounts of products, it is essential that the starter culture is able to develope and show enzymatic activity in the fat emulsion. Therefore a small amount of tryptone and yeast extract was added to the emulsion. This may contribute to small amounts of protein based aroma compounds.

Table 1 lists the identified compounds for reference samples as well as all starter cultures including the method of identification. Certain compounds are found universally in all samples. The origin of furfural and other furan derived compounds is probably limited hydrolysis of gum arabic during autoclavation of the fat emulsion. Some compounds are due to autoxidation of lipids. These include 1-butanol, aldehydes such as hexanal, heptanal, octanal, nonanal, and benzaldehyde. A greater amount of some of these compounds are observed in samples with starter cultures due to lipolytic action of the bacteria, generating free fatty acids. Aldehydes like hexanal, heptanal contribute to the flavor of meat products with a rancid odour.

Also a considerable amount of acids: acetic acid, 2-me-propanoic acid, butanoic acid, 3-me-butanoic acid and 2-me-butanoic acid are produced. These acids might be further converted to various esters. However only ethylacetat and methyl-hexanoate are found and these compounds are confined to lactobacilli strains. Esters are observed in products like ham and sausage and are belived to contribute significantly to the flavor of these products (Barbieri et al., 1992; Stahnke and Zeuthen, 1992).

Only few bacterial fermentation products are observed due to lack of readily fermentable carbohydrates in the substrate. 2,3-butanedione and 2,3-pentanedione as well as acetoin are observed for all cultures except *S. carnosus*. Acetic acid is only produced by the *Staphylococci* and *P.pentosaceus*. These compounds contribute with a "butter" odour.

Conclusion

As table 1 shows there is a considerable difference between the genus of bacteria and even between the two staphylococci species in the same genus. The aroma profile - the type and amount of volatile compounds produced by the lactobacilli are relatively similar. However great diversity exists between *S. xylosus* and *S. carnosus*. On the other hand *S. xylosus* and *P. pentosaceus* have many compounds in common; especially acid production is pronounced with these strains. Although the type of volatiles products may seem alike for the different bacteria, considerable difference does exist in the amounts of the individual compounds. The aroma

profile must be considered as a whole, and bearing in mind the different threshold values for volatiles in a sensoric evaluation, the "fingerprint" given by the gaschromatographic analysis could be an effective way of testing the capability for aroma formation from starter cultures.
Examples of chromatograms for *S. xylosus* and *L. sake* are given in figure 1 and 2.

References

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Captions:

Table 1. Identified volatiles from various meat starter cultures.

Figure 1. Chromatogram of volatiles produced by *S. xylosus*.

Figure 2. Chromatogram of volatiles produced by *L.sake*.