Protection and certification of typical meat products

GENERATION OF VOLATILE COMPOUNDS BY *STAPHYLOCOCCUS XYLOSUS, DEBARYOMYCES HANSENII,* AND *PENICILLIUM CHRYSOGENUM* DURING PORK RIPENING

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1. Background

The acceptability of dry cured meat products is closely related to flavour (García *et al.*, 1998). Different micro-organisms are able to produce meat products with increased flavour, including bacteria (Hinrichsen and Andersen, 1994; Sondergaard and Stahnke, 2002), yeasts (Olesen and Stahnke, 2000), and moulds (Bruna *et al.*, 2001). The most outstanding contribution of micro-organisms to volatile compounds in meat products consists of producing branched aldehydes and their respective alcohols, ketones, acids, and ethyl esters from branched chains amino acids, as well as decreasing lipid oxidation products (Hinrichsen and Pedersen, 1995; Montel *et al.*, 1998; Bruna *et al.*, 2001; Sondergaard and Stahnke, 2002). However, more complex reactions are devised as the variety of studied products increases (Sunesen *et al.*, 2001). Most of previous work on this field used selected strains that were inoculated into fermented products, but other micro-organisms, particularly lactic acid bacteria, were allowed to grow during fermentation. Given that complex interactions may take place when different micro-organisms grow together, the final outcome can change substantially with uncontrolled organisms present. In addition, very little work has been carried out on the volatile compounds generated from meat ripened under strict sterile conditions, as a control for changes due solely to micro-organisms. For this, a more basic approach can be very useful to ascertain the role of the microbial population on flavour generation, particularly in dry-cured meat products.

2. Objectives

The purpose of this work was to show the impact of combining different micro-organisms on the volatile compounds and flavour of ripened pork, further than a simple addition of individual metabolisms.

3. Materials and methods

Staphylococcus xylosus Sx5EA, *Penicillium chrysogenum* Pg222 and *Debaryomyces hansenii* Dh345 strains isolated from dry cured ham (Rodríguez y col., 1998) were used. Pork loins were sterilised by searing and cut into 0.5 cm thick slices. Samples (100g) were distributed in sterile flasks, salted (5g NaCl), inoculated with 3 ml of cultures, with c.a. 10^6 ufc/ml of the selected strains, and incubated (25ºC, 45 days). Sterile slices were incubated as controls. Samples were collected at 3, 7, 10, 15, 30 and 45 days. Growth of micro-organisms was evaluated by counting on MSA and MEA. Volatile compounds were extracted by Solid Phase Micro-Extraction technique with a poly dimethylsiloxane fibre (Ruiz *et al.* 1998) and identified by GC-MS. Compounds were tentatively identified by comparing their mass spectra with those contained in the NIST/EPA/NIH and Wiley libraries and by Kovats indices (Acree and Arn, 1997). Sensory analysis was carried out by a panel of 20 tasters trained in the sensory assessment of meat products. The acceptability of odour was assessed in a hedonic test using a non-structured scale (from 0= very poor, to 10= excellent) and the odour was identified in a description test.

4. Result and discussion

The micro-organisms inoculated showed a progressive increase, reaching about 10^8 cfu/ml in 15 days (Fig. 1).

Overall 60 volatile compounds were detected and identified, including 12 hydrocarbons, 5 alcohols, 10 ketones, 11 aldehydes, 9 acids, 6 esters, 2 furans, and 5 pyrazines.

Some aliphatic hydrocarbons attributed to **oxidative decomposition of lipids** were found in all the inoculated batches but not in sterile samples (Table 1). Similarly, linear aldehydes (C_5-C_9) were detected only at small quantities in the sterile batch, but at higher levels with *D. hansenii* alone and combined with the other organisms. However, these aldehydes were not even detected in batches with either *S. xylosus* or *P. chrysogenum*. Linear ketones also showed low levels of just 2-propanone and 2-heptanone in sterile samples. As for aldehydes, *D. hansenii* generated higher levels most C_3-C_9 ketones. On the other hand, *S. xylosus* and *P. chrysogenum* produced high amounts of ketones too. Surprisingly, samples inoculated with all micro-organisms showed a similar profile to sterile control with only 2-propanone and 2-heptanone. Both linear aldehydes and linear ketones derive from lipid oxidation that can be promoted by microbial ß-oxidation (Barbieri et al., 1992; Montel et al., 1998; Sunesen et al., 2001). Thus, lipid oxidation is rather limited in sterile samples but seems to reach higher levels with every organism tested. However, either *S. xylosus* or *P. chrysogenum* led to ketones, *D. hansenii* led to both aldehydes and ketones, and the three organisms together led mainly to aldehydes.

Different compounds associated to **amino acids metabolism** were detected (Table 1). Branched aldehydes 2-methylbutanal and 3-methylbutanal were not detected in sterile samples, or just 3-methylbutanal at moderate levels from one intermediate sampling with *S. xylosus*. These branched aldehydes showed a progressive increase along

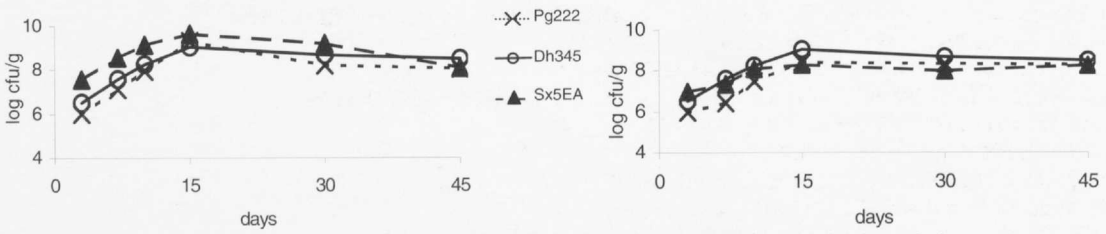

Fig. 1. Evolution of microbial counts in batches after individual (left) or combined (right) inoculation.

Table 1. Level of the main volatile compounds detected from the different batches.

	Sterile control	*S. xylosus*	*D. hansenii*	*P. chrysogenum*	Sx+Dh+Pc*
Hydrocarbons	-**	++	+++	+++	+++
Linear aldehydes (C_5-C_9)	+	-	+++	-	+++
2-propanone	++	+++	+++	+++	+++
2-heptanone	+	++	++	++	++
Other linear ketones (C_4-C_9)	-	+++	++	+++	-
2-methylbutanal	-	-	++	++	++
3-methylbutanal	-	+	++	++	+++
3-methylbutanol	-	-	-	+++	-
3-methyl-2-pentanone	-	+	+++	++	-
2-methylbutanoic acid	+	+	+	++	+
3-methylbutanoic acid	+	-	+	++	-
2-methylbutanoic acid ethyl ester	+	-	++	-	-
3-methylbutanoic acid ethyl ester	+	-	++	-	-
1-octen-3-ol	-	-	-	++	-
Ethanol	+++	+++	+++	+++	+++
Acetic acid	+++	-	++	+	+++
Pentanoic acid	-	+	++	-	+++
Other acids (C_3-C_{10})	-	-	-	-	+++
Pyrazines	-	+	++	+++	-
Furans	-	-	-	+	+++

* Batch inoculated with the three micro-organisms together.
** Level of total ion count: (-) not detected; (+) 10^5 - 10^6; (++) 10^7; (+++) 10^8 - 10^9.

ripening in the remaining batches. Branched acids were seldom found, except from the batch with *P. chrysogenum* at the last sampling. Similarly, ethyl esters of branched acids were detected only in sterile and *D. hansenii* batches. The ketone 3-methyl-2-pentanone, related to 2-methylbutanal, was found in all batches inoculated with just one strain, but not with the three strains. On the contrary, the only branched alcohol detected (3-methylbutanol) was found just with *P. chrysogenum*. The reduction of 3-methylbutanal to the corresponding alcohol is consistent with the antioxidative effect attributed to moulds (Lücke, 1998), but it was not observed when *P. chrysogenum* grew with other organisms. All these results indicate that the active metabolism of amino acids shown when the mould and the yeast grew individually can be overlapped by other micro-organisms present.

Among **other compounds** (Table 1), ethanol was obtained at every sampling from inoculated batches, but only after two weeks of incubation in the sterile batch. Its presence in sterile pork has to be due to autolytic reactions, which seem to be favoured by the microbial metabolism. Most carboxylic acids were detected only from inoculated samples. Just acetic acid was found in sterile samples. Several short chain fatty acids were detected from samples with the three micro-organisms together, but not in the individual inoculation. On the contrary, pyrazines were detected from batches inoculated individually with each organism, but not with all three together.

Flavour evaluation rated sterile control with the lowest score (4.4), while the highest acceptability was reached by the batch inoculated with the three micro-organisms (7.3), followed by *P. chrysogenum* (6.7), *D. hansenii* (6.2) and *S. xylosus* (6.1). However, only the batch with *P. chrysogenum* had flavour notes related solely to cured meat. In the remaining inoculated batches other notes to "cheese", "beer", "fruit", "butter" or "bread" were detected, while the sterile control was qualified as "rancid" or unpleasant.

5. Conclusion

The contribution of micro-organisms is essential to generate volatile compounds and flavour notes associated to cured meat in the conditions tested. The interaction of the three micro-organisms led to complex reactions that go much further than the results expected from the observations with individual strains. Thus, the precise composition of the microbial population on dry-cured meat products should be under strict control.

6. Pertinent literature

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