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EFFECT OF SELECTED MICROORGANISMS ON DRY FERMENTED SAUSAGE VOLATILES

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INTRODUCTION

During the ripening of dry fermented sausage occur several biochemical events determining the product final flavour. First, the carbohydrates are fermented by the lactic acid bacteria yielding mainly lactic acid although other acids (acetic, propionic, ...) may be also produced in smaller quantities (De Keteleare et al, 1974). Second, the nitrates and nitrites are reduced by the *Micrococcus* to nitric oxide which influences the final colour and flavour (Demasi et al, 1989; Brown et al, 1974). Third, the meat proteins and lipids are partially degraded by microbial flora and tisular enzymes which together with peroxides and oxygen generate many substances responsibles of sausage flavour (Demeyer et al.,1974; De Keteleare et al, 1974; Davidek et al., 1990). The sausage overall flavour is a combination of the different compounds produced during the fermentation and ripening by the above mentioned phenomena.

The objective of this work is an attempt to establish the contribution of the meat enzymes, *Lactobacillus*, *Micrococcus* and oxidative reactions to the flavour compounds generation.

EXPERIMENTAL METHODS

Sausages preparation: M. semitendinosus (beef) and M. longissimus dorsi (pork) were removed from the carcases at 48 hours *post-morten* and the exterior surface of the muscles was sterilized by searing. Then, the burnt tissues were removed down to a depth of about 3mm, using sterile instruments in a laminar flow cabinet. The skin of lard (6-8 cm thick) from Iberian pig was sterilized by the same method. The subcutaneous layers of lard fat were removed with sterile instruments throught an incision in the burnt skin. Solutions of glucose and lactose were sterilized by filtering (0.45 µm); phosphates, NaCl, nitrates and nitrites were autoclaved and then concentrated by lyophilisation. All operations of sausage manufacture were performed in a laminar flow cabinet and the operators used sterile surgery masks and gloves. Meat and fat were comminuted in a autoclaved manual grinder. The ingredients were aseptically prepared to give the final composition (%): pork 40; beef 40; fat 13; glucose 1.5; lactose 0.5; phosphates 0.3; NaCl 2.5; $NaNO_2$ 0.1; KNO_3 0.2 and sterilized water to dissolve the lyophilisated ingredients. No species were added.

Four batches of sausages were made. The first was inoculated with *Lactobacillus plantarum* 4045; the second with *Micrococcus-12*;. the third with *Lactobacillus plantarum* 4045 and *Micrococcus-12;* the fourth batch was not inoculated, being the aseptic batch. The final mixture was filled into artificial casing soaked (three days) in sterile 10% saline solution and washed just before use in plenty of sterile distilled water to minimize contamination. About 500g of every sausage batch were prepared and ripened in ripening cabinet (Kowell Mod. CC3AFY) programmed 48 h at 22℃ and relative humidity (RH) 90%; thereafter 30 days at 12℃ andRH 85%.

Analytical procedure

Portions of sausages (25g) were coarsely ground and placed in a conical flask (500 ml) where samples were allowed to equilibrate at room temperature for 30 min. The flask was then equipped with a Dreschel bottle head and a glass trap (70 mm long x 2 mm o. d. x 1.5 mm i. d.) packed with 8 mg Tenax GC 80-100 mesh attached via a screw joint on the head outlet. During collection, the system was mantained at 29℃. Volatiles were swept onto the trap using a nitrogen flow (80 ml/min) for exactly 10 min. The sample was thermally desorbed using a Perkin-Elmer PTV by increasing ballistically (150℃/s) the injector temperature to 300℃ and maintaining it there for 5 min. The chromatographic analysis was performed in a Perkin-Elmer 8420 Gas Chromatograph. A 50 m x 0.25 mm i.d. fused silica capillary column coated with a cross-linked 0.25 µm film of FFAP was used. The column temperature was initially maintained at 55℃ for 10 minutes and then programmed at 2℃/min to 100℃ remaining 10 minutes at 100℃, then a rate of 10℃/min up to 180℃ and the final temperature was maintained 25 minutes. Identification of peaks was performed by GC-MS using a Perkin-Elmer ITD-50 Ion Trap Detector (electronic impact 70 e. v.). Compounds were tentatively identified by computer comparison of spectra with those of the NBS library. Identification of some of these compounds was subsequently confirmed by matching their spectral data with those of authentic reference compounds analyzed under identical conditions.

RESULT AND DISCUSSION

More than 30 different compounds were detected in the experimental sausages, including aldehydes, ketones, alcohols, hydrocarbons and short chain fatty acids. In some samples, several of the above mentioned volatile compounds were detected in trace amounts. The headspace volatiles of the four batches contained essentially the same substances (Table 1) although there were relative differences among the sausages. The relative contribution of some selected volatile compounds to the headspace of each sausage type is shown in Table 1. The compounds selected for comparison among sausages were only those accurately characterized. The selected volatiles included aldehydes (9), ketones (3), alcohols (2), hydrocarbons (3) and short chain fatty acids (3).

The number and concentration of volatile compounds in the experimental sausages were always less than those found, using the same methodology, in the same type of commercial sausages (unpublished data). This fact may be explained by both, a minor contribution of the microbial flora and to the lack of black pepper which has a great contribution in the overall sausage volatile profile of this kind of sausages (Debrauwere and Verzele, 1976). Many of the here detected compounds have been described by other authors

(Berger et al., 1990; Edwards et al, 1991; Stahnke et al., 1992; Berdagué et al., 1993). The observed differences in the four batches were mainly due to the concentrations of volatile compounds rather than to the types of volatiles. In general, the volatile concentration of a particular compound increased along ripening reaching at 26 days values 2-4 fold higher than those at 0 days. No clear differences were observed between batches, including the aseptic one. This fact allows hipothetized that lipid autooxidation was the prevalent phenomenon because the main differences on volatile concentrations along ripening affected the aldehydes which are mainly formed via lipid oxidation (Frankel, 1983).

It has been reported that 3-hydroxybutan-2-one and acetic acid are generated by fermentation pathways (Kandler, 1983) and 3-methylbutanal and 3-methylbutan-1-ol are derived from the catabolism of branched aminoacids (MacLeod and Morgan, 1958). Indeed, these substances excepting 3-methylbutanal showed the major differences when results of inoculated batches and the aseptic one are compared.

Several esters have been detected (Edwards et al.,1991) in commercial sausages (salami and salchichón). These authors have associated he presence of these substances in sausages with microbial activities. The lack of these substances in the experimental sausages supports again the relevance of autooxidation reactions. Probably, if the ripening period had been longer the esters might have been detected.

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TABLE 1. Relative changes of selected volatile compounds in experimental dry sausages during ripening

Batch	aseptic				L. plantarum 4045			Micrococcus-12			L. plantarum 4045+ Micrococcus-12		
	Days of ripening												
COMPOUND	0	2	5	26	2	5	26	2	5	26	2	5	26
3-methylbutanal	1+	1+	1+	2+	1+	1+	2+	1+	2+	1+	1+	1+	2+
hexanal	T	1+	1+	2+	1+	1+	2+	1+	1+	1+	1+	1+	2+
2-hexenal	T	1+	1+	2+	1+	1+	2+	1+	1+	2+	1+	2+	2+
octanal	1+	1+	1+	2+	1+	1+	2+	1+	1+	2+	1+	2+	2+
nonanal	1+	2+	1+	3+	1+	1+	3+	2+	2+	3+	2+	2+	3+
decanal	2+	2+	2+	2+	2+	1+	2+	2+	2+	3+	2+	T	T
benzaldehyde	2+	2+	2+	3+	2+	2+	2+	3+	2+	4+	4+	3+	3+
unidentified aldehyde 1	2+	4+	3+	5+	4+	4+	5+	4+	4+	5+	4+	5+	5+
unidentified aldehyde 2	2+	3+	3+	5+	3+	3+	4+	4+	4+	5+	4+	5+	5+
3-hydroxybutan-2-one	1+	2+	2+	2+	1+	1+	2+	1+	2+	3+	1+	3+	3+
pentan-2-one	T	1+	1+	1+	1+	1+	1+	1+	1+	1+	1+	2+	2+
butan-2-one	T	2+	1+	2+	2+	1+	1+	2+	2+	2+	2+	2+	2+
3-methylbutan-1-ol	T	1+	1+	1+	1+	1+	1+	1+	3+	2+	1+	2+	2+
pentan-1-ol	1+	1+	1+	1+	1+	1+	2+	1+	1+	1+	1+	1+	1+
n-heptane	T	T	T	1+	1+	1+	1+	T	T	T	1+	1+	1+
1,2 -dimethylbenzene	1+	1+	1+	2+	1+	2+	1+	1+	2+	1+	1+	1+	2+
unidentified alkylbenzene	1+	1+	1+	2+	1+	1+	1+	1+	1+	1+	1+	1+	1+
acetic acid	1+	2+	2+	3+	3+	4+	3+	3+	5+	3+	3+	5+	3+
butanoic acid	T	1+	T	T	T	T	T	T	1+	T	T	T	T
hexanoic acid	1+	2+	1+	2+	1+	1+	2+	2+	2+	3+	1+	2+	3+

Range of Peak Height in Analogue to Digital Converter Counts: T (Trace); 1+ (<10000); 2+(10000-20000); 3+(20000-30000); 4+ (30000-40000) and 5+ (>40000)