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EFFECTS OF ENVIRONMENTAL FACTORS ON LIPOLYTIC AND PROTEOLYTIC ACTIVITIES OF STARTER CULTURES IN MEAT-FAT MIXTURES

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INTRODUCTION

Starter cultures are used in the production of fermented meat products in order to shorten the ripening period, ensure colour development, enhance the flavour and improve product safety (Lücke and Hechelmann, 1985). The genera of bacteria successfully utilised as starter cultures are Lactobacillus, Pediococcus and Micrococcus/Staphylococcus. The lipolytic and the proteolytic activity of Micrococcus sp. and Staphylococcus sp. may contribute to the flavour of fermented meat products (Lücke and Hechelmann, 1985). Lactic acid bacteria may also possess weak lipolytic (Nielsen and Kemner, 1989; Molina *et al.*, 1991) and proteolytic (Law and Kolstad, 1993) activity, although the contribution of these activities to flavour is not well studied.

The metabolism of microorganisms is affected by environmental factors, thereby influencing their properties as starter cultures. Oxygen, pH and water activity are reported to affect product patterns (Rhee and Pack, 1980: Troller and Stinson, 1981; Borch and Molin, 1989). The environmental conditions vary during the ripening process with respect to pH, salt concentration, water activity, temperature and availability of oxigen and nutrients (Rödel, 1985; Nychas and Arkoudelos, 1990; Roca and Incze, 1990). Thus, ample possibilities exist for variation in the end product formation of the bacteria due to the ripening conditions applied.

The objective of the present study was to develop a method for preparing sterile meat-fat mixtures and evaluate the effects of environmental factors on proteolysis and lipolysis by bacteria and endogenous enzymes. The effects of oxigen availability, temperature, water/fat content, concentration of salt in the water phase and initial pH were evaluated using factorial experimental design and analysis of covariance.

MATERIALS AND METHODS

The bacteria used were Lactobacillicus pentosus SMRICC 359 (SMRICC, Swedish Meat Research Institut Culture Collection), Pediococcus pentosaceus SMRICC 360 and Staphylococcus xylosus SMRICC 361. All the strains originated from commercial starter cultures. The inocula were prepared by growing L. pentosus for SIX hours and P. pentosaceus for seven hours in APT broth (BBL, Cockeysville, Maryland, USA), and S. xylosus for seven hours in Trypton Soya Broth (Oxoid, Basingstokes, UK) at 30°C. At the end of the logarithmic growth phase, cells were harvested by centrifugation and resuspended in skim milk, frozen and stored at -80°C. After thawing, the sterile meat-fat mixtures were inoculated to a level of 10cfu/g.

The sterile meat-fat mixtures were composed of (w/w): lean pork, pork back fat, water and NaCL was varied in order to obtain a water/fat ratio and concentration of salt in the water phase in accordance with the factorial experimental design (Table 1), whereas the addition of glucose, $NaNo^2$ and Na-ascorbate was constant. In order to prepare sterile meat and fat tissues, the exterior of a pork loin was sterilised by searing using a gas-burner. Rind, surface tissues and bone were removed using sterile instruments. The remaining meat and fat were cut into pieces and minced twice through a 6mm chopper disc. All operations were carried out under sterile conditions on a sterile bench. The sodium

chloride was dry-heat sterilised and the glucose solution was sterilised by autoclaving. Solutions of nitrite and ascorbate were filter sterilised.

The storage experiments were carried out in a randomised order using fractional factorial experimental design (Table 1; Box *et al.*, 1978). The sterile meat-fat mixture was divided into four parts. Three of the portions were inoculated with a culture of either L. pentosus, P. pentosaceus or S. xylosus. The fourth portion was not inoculated. The pH was adjusted with sterile 1/2M HCL or 1M NaOH. Samples of 100g of the meat-fat mixture were stored as a 1cm thick layer in glass jars (10cm in diameter, 5cm in height). A loose fitting lid was used where the atmosphere could freely diffuse into the jar. When stored aerobically, the jars were wrapped with an oxigen permeable polyethylene film. Anaerobic atmospheres were established by packaging the jars in gas impermeable pouches (Lamofoil 4 ply, Otto Nielsen Ltd, Lyngby, Denmark). The pouches were evacuated and then filled with a sterile filtered gas mixture of 95%N^2 and 5% CO^2.

At 0 and 19 days of storage, the samples were analyzed for bacterial growth on APT agar (BBL, Cockeysville, Maryland, U.S.A). incubated at 25°C for 3 days, NPN (DeMasi *et al.*, 1990) and FFA (Anon, 1954). The effects of oxigen, temperature, water/fat ratio, concentration of salt in water phase and initial pH on the formation of FFA and NPN were evaluated by analysis of covariance with normalised values. (Wilkinson, Leland. SYSTAT: The system for Statistics. Evanston,IL. U.S.A.: SYSTAT,Inc.,1990). No interaction effects were included in the models, as the factorial design was maximally reduced.

RESULTS AND DISCUSSION

The inoculation of a sterile model system with a pure culture of bacteria makes it possible to obtain information on metabolic activities specific to a single bacterial species. A sterile control of the meat-fat mixture is essential in order to conclude whether the chemical changes are due to the enzymatic activities of the meat/fat tissues or to the activities of the added bacteria. In the present study, a method for preparing sterile meat-fat mixtures was successfully developed. In all of the experiments performed, the uninoculated control meat-fat mixtures remained sterile throughout storage. A similar sterile system has not been reported earlier.

Lipolysis was indicated both in the inoculated meat-fat mixtures and in the uninocultated control by the increase of FFA (Figure 1). In several experiments, there were only small differences in lipolysis between the uninoculated control and the meat-fat mixtures inoculated with the different bacteria. However, in others there was more lipolysis in the inoculated meat-fat mixtures with some differences between the bacteria. The tendency towards higher levels of FFA in some of the meat-fat mixtures inoculated with L.pentosus, P.pentosaceus or S.xylosus, as opposed to the control, indicates bacterial lipolytic activity. P.pentosaceus and S.xylosus are reported to have lipolytic activity on pork fat (Nielsen and Kremner, 1989; Molina *et al.*, 1991.)

The formation of FFA was significantly affected by the temperature for L.pentosus and P.pentosaceus (Figure 2). In both cases, most FFA was formed at high temperature. On the other hand, none of the environmental factors significantly affected the increase of FFA in the uninoculated control or meat-fat mixtures inoculated with S.xylosus.

Proteolysis was indicated both in the inoculated meat-fat mixtures and in the uninoculated control by the increase of NPN (Figure 3). The highest increase of NPN was shown by L.pentosus in many of the experiments. Also, P.pentosaceus showed a higher increase of NPN than S.xloysus or control. The higher NPN found in some of the meat-fat mixtures inoculated with L.pentosus and P.pentosaceus as opposed to the control indicates bacterial proteolytic activity under certain environmental conditions.

The formation of NPN was significantly affected by the temperature for the control (Figure 4). Most NPN was significantly affected by the oxygen availability, the temperature and the salt concentration. Most NPN was formed anaerobically, at high temperature and low salt concentration. For S.xylosus the formation of NPN was significantly affected by the oxygen availability and the temperature. Most NPN was formed anaerobically at high temperature.

The NPN and FFA fractions are known to increase due to proteolysis and lipolysis during ripening of fermented

sausages. NPN concentrations of 9% of total nitrogen in the beginning and up to 22% at the end of ripening have been observed (Lois *et al.*, 1987; Astiasaran *et al.*, 1990). FFA contents between 3% and 8% of the total fatty acids at the end of ripening have been reported (Lois *et al.*, 1987; Lizarrage *et al.*, 1989). This is similar to the results of the present study, in which the content of NPN increased from 10 to 27% and the content of FFA reached 9% at most. Thus, the storage of meat-fat mixtures probably reflects the biochemical changes occurring in a fermented sausage during ripening.

CONCLUSION

A method for preparing sterile meat-fat mixtures was developed. Lipolysis and proteolysis were shown by endogenous enzymes as well as by bacteria, L.pentosus, P.pentosaceus and S.xylosus. Lipolysis was affected by the temperature and proteolysis by the oxygen availability, temperature and salt concentration.

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Table 1. The fractional factorial experimental design with five variables. Oxygen was studied at two levels, anaerobic (-) and aerobic (+) and temperature, water/fat ratio, concentration of salt in the water phase and initial pH at three levels, low (-), high (+) and centre points (0). Intended values of each variable are given in brackets.

Exp. #	Oxygen	Temp. (°C)	Water/fat ratio	Salt (% in water)	Initial pH
1	-	-(18)	-(40/40)	+(6.5)	+(6.0)
2	+	-(18)	-(40/40)	-(2.9)	-(5.0)
3	-	+(30)	-(40/40)	-(2.9)	+(6.0)
4	+	+(30)	-(40/40)	+(6.5)	-(5.0)
5	-	-(18)	+(50/30)	-(2.9)	-(5.0)
6	+	-(18)	+(50/30)	-(2.9)	+(6.0)
7	-	+(30)	+(50/30)	+(6.5)	-(5.0)
8	+	+(30)	+(50/30)	-(2.9)	+(6.0)
9	-	0(24)	0(45/35)	0(4.5)	0(5.5)
10	+	0(24)	0(45/35)	0(4.5)	0(5.5)