PROTEOLYSIS AND LIPOLYSIS IN AN ASEPTIC MEAT MODEL SYSTEM FOR DRY SAUSAGES

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Background

Fermented meat products are produced through a complex series of microbial, physical (drying, gel formation,...) and (bio)-chemical changes (proteolysis, lipolysis, colour formation,...) and their interactions. The overall quality of a fermented sausage is determined by the balance of these changes depending on processing conditions, starters and raw material properties (*Demeyer et al., 1986; Molly et al., 1997; Montel et al., 1998*). A detailed knowledge of such changes would allow a better standardisation and optimisation of the quality and safety of the fermented meat product. Recent data suggest that in the first stages of the fermentation process endogenous proteolysis and lipolysis by animal tissue enzymes are more important than bacterial metabolism (*Verplaetse et al., 1989; Molly et al., 1997*). As muscle enzyme activity may vary with carcass and raw meat characteristics (*Uyterhaegen et al., 1994; Toldrá et al.,1996*), the latter may be important for development of sausage quality.

Objectives

1. Development of an aseptic meat model system (AMMS) to simulate the initial stage of the fermentation process
2. Evaluating the effect of different pork carcass meat percentages on initial proteolytic and lipolytic activity in an AMMS

Methods

Development of an AMMS

Twenty Belgian Landrace Negative × Piétrain crossbred pigs with an average live weight of 101 kg (SD 3) were slaughtered. Carcass meat percentages varied from 50 % to 68 %. The group consisted of 10 barrows and 10 gilts. The exterior of the m. *triceps brachii* of the right carcass half was burned off with a portable blowtorch (Laser camping, oxyturbo, Italy). Then, surfaces were removed using sterile knives and the remaining meat was divided into thick slices and vacuum-packed stored at -18°C. All the meat was stored frozen for exactly 4 months. Before utilisation, each frozen muscle was cut into cubic blocs of 3 to 5 cm and randomised. A representative meat sample with 1 % of glucono-delta-lactone (GDL), 3 % of colouring salt (NaCl containing 0.6 % $NaNO_2$) and a mixture of antibiotics was minced for 1 min in a sterilised mincer (maxi 300 chopper, SEB, Dijon, France) (soaked for 24 hours in a mixture of ethanol and acetic acid (3/1 v/v)). GDL was added as a chemical acidulent to compensate for absent microbial acid production and corresponding pH drop. The addition of 1000 U penicillin G (continental Pharma), 200 µg amphotericin B (Bristol-Myers Squibb) and 1 mg streptomycin sulfate (Sigma) per g mixture was necessary to inhibit microbial growth, mainly yeasts. Meat mixtures of 50 g were vacuum packed into polyethylene bags and incubated in duplicate for 3 days at 25°C. Under these circumstances, an average pH of 4.8 was achieved comparable with the final pH after a Northern fermentation process (*Molly et al., 1997*).

Methods of analysis

Total cathepsin D activity, important in proteolysis during dry sausage fermentation (*Verplaetse et al., 1989*), and acid lipase activity were determined on the m. *triceps brachii* by a modification of the procedures described by respectively Barret and Kirschke (1981) and Motilva and Toldrá (1993). Before and after incubation, the following analyses were performed on the meat mixtures: total viable aerobic and micro-aerophilic counts were measured on Plate Count Agar. Proteins were separated using SDS-PAGE as described by Greaser et al. (1983). Free non-protein α-NH_2-nitrogen was extracted with 0.6 M $HClO_4$ and determined by a method of Oddy (1974). Total proteolytic activity was evaluated by analysing the total tryptophan content in the non-protein nitrogen fraction according to Messineo and Musarra (1972). Ammonia contents were determined using a 692 pH/ionmeter equipped with an ammonia selective gas electrode (Metrohm, Herisau, Switzerland). Free fatty acids (FFA) were extracted with a chloroform/methanol (2/1) mixture (*Folch et al. 1957*) and determined according to Koops and Klomp (1977).

Results and discussion

Total aerobic and aerophilic micro-organisms measured after incubation were below 10^3 CFU/g mixture, showing that bacterial activity is absent for all practical purposes. The results of the analyses are shown in Table 1. The SDS-PAGE profiles of the AMMS were compared with those after 3 days industrial dry sausage fermentation (*Verplaetse, 1994*) and both showed the same pattern but differed in band intensities. The absence of bacterial activity and use of younger meat, stored for relatively short periods (*Henahan et al., 1981*), may explain this difference. Low but significant amounts of ammonia produced reflect both the absence of bacterial activity as well as suggesting endogenous activity.

Correlations between proteolytic parameters and carcass meat percentage were calculated but only a positive significant correlation between carcass meat percentage and cathepsin D activity was obtained ($r = 0.52$, $p < 0.05$). According to Figure 1, this correlation was mainly caused by significant differences in both carcass meat percentage and cathepsin D activity between the 2 sexes ($p < 0.05$). A positive trend between both parameters, mainly for barrows, can be observed. Valin and Ouali (1992) also showed that different meat species show a clear variability in activity of proteases and their inhibitors as well as in susceptibility of proteins to proteolysis.

No significant correlation was found between acid lipase activity, FFA production and carcass meat percentage. The considerable variation observed in the end products of incubation (Table 1) should therefore be explained by uncontrolled factors as genotype, animal

diet, growth rate and age of the animals. It should also be realised that variability in rates of pH drop in the incubation also has affected enzyme activities.

Conclusions

-The developed aseptic meat model system (AMMS) is an interesting tool to simulate animal tissue activities in the initial stage of the fermentation process. - Gilts have significant higher carcass meat percentages and cathepsin D activities in m. *triceps brachii* than barrows. - Differences in pork carcass meat percentage of the animals used cannot explain considerable variability in initial proteolysis and lipolysis in the AMMS.

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Table 1 Mean value and standard deviation (SD) for cathepsin D activity and acid lipase activity in m. *triceps brachii*, pH and produced amounts of ammonia, non-protein free α-NH_2-N, non-protein tryptophan, FFA and SDS-PAGE protein fractions in the AMMS

	Mean	SD
Cathepsin D activity (U/g fresh matter)	37.2	6.3
Acid lipase activity (U/g fresh matter)	7.3	1.0
pH	4.7	0.1
Ammonia (mg N /g N)	1.01	0.51
Non-protein free α-NH_2-N (mg N /g N)	5.65	1.40
Non-protein tryptophan (mg /g N)	1.21	0.18
FFA (mg palmitic acid /g fat)	16.4	9.8
SDS-PAGE profile (µg BSA-equivalents / mg SDS-soluble meat protein)		
myosin heavy chain	12.7	4.9
heavy meromyosin (HMM)	-13.3	4.3
actin	14.3	3.6
38 kDa protein	-23.8	3.3
sum of increasing peaks*	-45.2	6.1
sum of decreasing peaks*	66.7	11.8

* is the sum of the clearly visible increasing peaks
** is the sum of the clearly visible decreasing peaks

Figure 1 Cathepsin D activity (U/g fresh matter) measured in m. *triceps brachii* in relation to carcass meat percentage of barrows (□) and gilts (◆)