

## EFFECT OF MEAT SPECIES ON PROTEOLYSIS DURING DRY SAUSAGE FERMENTATION

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### SUMMARY

Proteolysis and fermentation was investigated in all meat sausages prepared consecutively from frozen horse, pork and beef, but using the same fermented sausage as inoculum. Samples were taken for determination of pH, lactate, myosin and actin ( SDS-PAGE ), free and peptide bound  $\alpha$ -NH<sub>2</sub>-N, ammonia and cathepsin D activity 0, 1, 2, 3, 7, 14 and 21 days after filling. The results indicate that total exo- and endopeptidase activity is equivalent to 400, 249 and 243  $\mu$ moles of released N per Kg DM in horse, beef and pork sausages respectively. In horse sausages, and, to a less extent in beef sausages, more actin and myosin degradation and higher cathepsin D activity were observed than in pork sausages. Differences are apparent, mainly during the fermentation period. Such differences may be related to the slight differences observed in amounts of lactate produced as well as to the longer lag time of lactate production observed for horse meat and, to a less extent, for beef, compared to pork.

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### INTRODUCTION

In an accompanying paper ( Verplaetse et al, 1992 ), we have shown that proteolysis during fermentation and drying of dry sausage involves the collaborative and consecutive action of both muscle and bacteria proteases. Muscle proteinases ( endopeptidases ) of the cathepsin D type, are mainly active during fermentation, whereas both bacterial and muscle exopeptidases, are mainly active during drying. In recent work ( Demeyer, 1992 ), we have shown that concentrations of non protein N (NPN) fractions in fermented dry sausage correlate very well with taste panel flavour evaluation. In fact, the data suggest that that proteolysis may be more important for flavour development than lipid and carbohydrate metabolism. This finding is in line with the significant rôle of free amino acids and oligopeptides in the development of meat taste during storage ( Nishimura et al, 1988 ) and with the rôle of both bacterial and endogenous proteases in development of cheese texture and flavour ( Fox, 1989 ). It can thus be assumed that eventual differences in meat proteinase activity may result in development of different sausage flavours, as earlier suggested by Fournaud ( 1976 ). Differences in meat species proteinase activity may exist, analogous to differences in the calpain/calpastatin system observed by Ouali & Talmant ( 1990 ). Such differences should then be reflected in different patterns of proteolysis in fermented sausages, prepared from different meat species but using the same inoculum. We have tested this assumption using fermented dry sausage, produced from horse, beef and pork meat.

## MATERIALS AND METHODS

**Sausage preparation** : All meat sausage batters ( 10 kg ) were prepared consecutively from frozen horse, pork and beef, using a 3d. old sausage mix ( 1% ) as starter. Sausages ( 90mm, ca. 1 Kg ) were fermented and dried up to 21 days after filling. Formulation, fermentation and drying were exactly as described in the accompanying paper, with the omission of glucono-delta-lactone and of pork back fat.

**Sampling and Analyses** : Samples were taken for determination of pH, lactate, myosin and actin ( SDS-PAGE ), free and peptide bound  $\alpha$ -NH<sub>2</sub>-N, ammonia and cathepsin D activity 0, 1, 2, 3, 7, 14 and 21 days after filling. Sample extraction and techniques used were referred to or described in the accompanying paper ( Verplaetse et al, 1992 ). Total cathepsin D activity was measured as described by Barrett & Kirschke ( 1981 ), but based on tryptophane determination ( Messineo & Mussarra, 1972 ) using hemoglobin as substrate and expressed as activity units per g of crude protein.

## RESULTS AND DISCUSSION

Table 1 shows that after the fermentation period ( 3 days ), increasing amounts of carbohydrate were fermented in the sequence horse, beef and pork sausage. This order was reflected in the values for lactate production. Differences were less apparent after drying up to 21 days. These differences were slight however, compared to those in NPN production where, nearly twice as much was formed in horse sausages compared to beef. Pork sausages showed less NPN production than

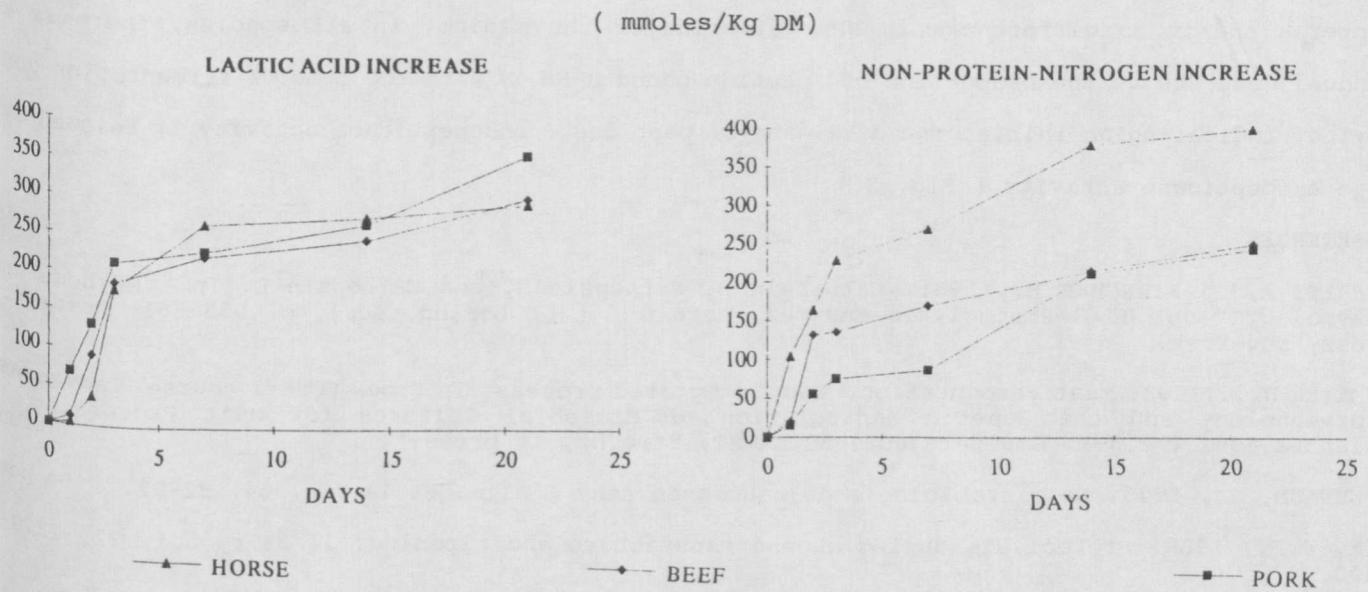
Table 1. Effect of meat species on sausage fermentation and proteolysis <sup>1</sup>.

	After fermentation (0-3days)			After drying (0-21 days)		
	Horse	Beef	Pork	Horse	Beef	Pork
pH	5.2	5.3	5.4	4.9	4.9	4.9
Products Formed <sup>1</sup>						
carbohydrates	77	83	100	161	146	143
Lactate	178	187	214	292	300	356
Total NPN <sup>2</sup>	229	136	74	400	249	243
% Degraded Myosin	63	54	53	95	86	74
Actin	33	23	28	74	60	58
Cathepsin D activity <sup>3</sup>	165	117	118	158	110	105

<sup>1</sup> mmoles/Kg DM <sup>2</sup> free + bound  $\alpha$ -NH<sub>2</sub>-N + ammonia N <sup>3</sup> units/g crude protein

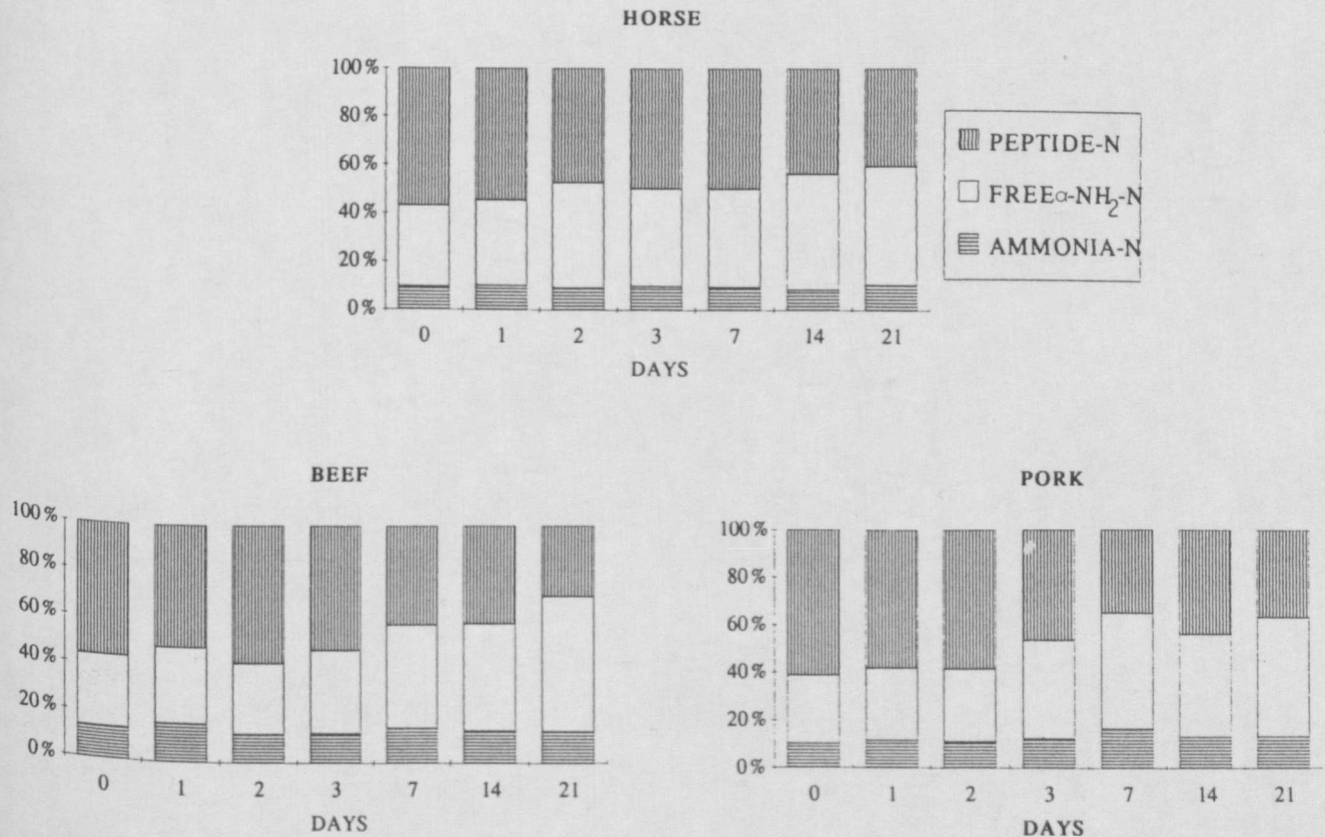
beef sausages, especially in the fermentation period. That such differences are related to differences in endogenous proteolytic activity is suggested by the species effect on myosin and actin degradation, as well as on cathepsin D activity. In both cases horse sausages showed the highest values, whereas values were somewhat higher for beef compared to pork sausages. Amounts

Fig.1. Species differences in kinetics of lactate and NPN production during dry sausage ripening



of NPN formed were higher than in earlier work ( Demeyer, 1992 ), obviously because of the all meat nature of the product. The differences in proteolytic activity described here may be related to differences in flavour development, associated with meat species differences. They may also be related to slight but clear differences in the kinetics of carbohydrate metabolism: Fig. 1 clearly shows that a longer lag time for lactate production is observed for horse

Fig.2. Species differences in the composition of NPN at different stages of sausage ripening.



sausages, whereas no lag time is observed for pork and an intermediate value is observed for beef sausages. Such effects may be related to differences in provision of NPN compounds for bacteria and/or to differences in NPN buffering of the medium. In all species, there is a gradual decrease in the proportion of peptide bound  $\alpha$ -NH<sub>2</sub>-N with the time of fermentation and drying, indicating an initial rapid release of peptides ( endopeptidase activity ), followed by more exopeptidase activity ( Fig. 2 ).

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