PROTEOLYSIS DURING DRY SAUSAGE RIPENING

VERPLAETSE,1,3 A., DE BOSSCHERE, M. and DEMEYER,1,2 D.

1 Onderzoekscentrum voor Voeding, Veeteelt en Vleestechnologie, University of Ghent, B-9230 MELLE, Belgium.
2 Instituut voor Biotechnologie, Vrije Universiteit, Brussels, Belgium.
3 Present address : Ter Beke N.V. Waarschoot, Belgium.

INTRODUCTION

The typical flavour of dry sausages is due to lactic acid, salt and a series of end products which originate from microbial & non-microbial (e.g. endogeneous meat enzymes) breakdown of carbohydrates, proteins & lipids. In this paper the proteolysis of meat proteins occuring during dry sausage ripening is studied using SDS-PAGE electrophoresis. An effort is made to identify the nature of the enzymes responsible for this breakdown from the degradation pattern.

MATERIALS & METHODS

PREPARATION OF SAUSAGES

The sausages were prepared in two commercial meat plants. The composition of the dry sausage mixture is given in table 1. Both meat and fat were ground and thoroughly mixed with the other ingredients in a bowl chopper . The resulting mixture was stuffed in casings (Naturin diameter 90 mm) and the sausages (ca. 2500 g) were fermented during about 72 h. at 22° C and 95 % relative humidity (%RH), after which they were transferred to a drying room for 18 days at 15° C and 85 % RH. During the fermentation period the sausages were daily smoked for ca 45 minutes.

SAMPLING PROCEDURE

Sausages from each plant were collected at 0,1,2,3,6,13 and 21 days after stuffing and immediately transported to the laboratory. A sufficient amount of the sausage was ground in a commercial meat grinder after removing the casing. The mixture was either used immediately or was vacuum packed in plastic bags and stored at -18° C.

PREPARATION OF MUSCLE EXTRACTS

Myofibrillar proteins were isolated from ca. 10 g. of sample according to the procedure described by Parrish et al (1973) using an Ultra Turrax (Janke & Kunkel KG - Staufen). Isolated myofibrils were dissolved overnight in imidazole buffer (0.0IM) (pH=7.0) containing 2 % sodium dodecyl sulfate (SDS) and 2 % Mercapto-ethanol (ME). Solutions were then filtered to remove connective tissue. After addition of bromophenol blue (ca. 3mg/10ml) and ca. 1,5 gr. sucrose/10ml the solution is frozen and preservated at -18° C until electrophoresis.

SDS-PAGE ELECTROPHORESIS

Electrophoresis was carried out according to the method described by Buts et al (1986) This method was slightly modified to get a good resolution of the different proteins present in the dry sausage extracts. Electrophoresis was performed in an Acrylophor model 144 apparatus (Pleuger, Antwerp) using gel rods of 15 cm length and 6 mm diameter. Gels were always prepared the day before use. All gels contained 0,55 % SDS and 0,50 % ME. For the separation of the high molecular weight proteins (>100.000 D) gels were used with a total acrylamide concentration (%T) of 5 % and cross linking (%C) of 3 %. Proteins with molecular weight varying from a 100.000 D to 20.000 D were separated on more concentrated gels (T=8 % and C=3%) Polypeptides present in dry sausage extracts with molecular weights lower than 20.000 D were differentiated on gels with 12 % T and 3 % C.

For the sample of day 0 20 µl of extract was applied on top of the gel. For the other samples the amount of drying of the sausage was taken into account, so that everytime the same amount of protein was subjected to electrophoresis.
The running, fixation, staining and destaining of the gels was done according to the method of Buts et al. Finally the gels were scanned using a Beckmann (model R.112) densitometer.

RESULTS

In both meat plants a similar pattern in protein breakdown was observed. During the fermentation and drying period the meat proteins were gradually degraded by proteolytic enzymes.
As can be seen in figure 1 degradation of myosin, actin and troponin T occured clearly during the production of dry sausages. Our study indicated that the heavy chain of myosin was broken down to a polypeptide with a molecular weight in the range 120.000 D - 150.000 D. This result was in agreement with previous studies reported by Penny (1980) and Bandman (1988) describing cathepsin activity. Proteolysis of meat proteins also causes an increase in the concentration of polypeptides with a molecular weight varying from 14.000 D to 36.000 D. The relative increase or decrease in protein concentration is given in table 2. These data show that during the fermentation process the heavy chain of myosin is degraded for ca. 49 %. Actin and Troponin T concentration were decreased by ca. 30 %. The low molecular weight polypeptides are increased by 75,9 % during the 21 day production.

CONCLUSION

These preliminar results show that the proteolytic pattern observed during dry sausage ripening is similar to the one produced by endogeneous cathepsins and is not influenced by the environment of the meat plant. This suggests that protein degradation in dry sausage is caused either by endogeneous meat enzymes or by bacterial proteases similar in activity to the cathepsins B & D or by both.
It may be significant that the sausage environment is optimal for the activity of cathepsins or cathepsin-related enzymes, because of a low pH (4.8-5.0) and a relatively high temperature (15° C - 22° C).
Proteolysis during dry sausage ripening may be a prolongation and intensification of meat conditioning and may have an important influence on flavour development.

REFERENCES

Bandman, E. & Zdanis, D. (1988). Meat Sci, 22,1.

Buts,B.,Claeys, E.& Demeyer,D.(1986). Proc. 32nd European Meeting of Meat Research Workers, Ghent, pp 175-178.

Parrish, F.C.,Young, R.B.,Miner, B.E.& Andersen,L.D. (1973). J. Food Sci., 38, 690.

Penny,I.F. (1980). The enzymology of conditonning. In Developments in Meat Sci. 1, Applied Sci. Publ., London, pp. 115-143.

Table 1 : Composition of the dry sausage mixture (% W/W)

ingredient	amount
lean pork	30,60 %
lean beef	30,60 %
pork back fat	35 %
salt	2,85 %
glucose	0,70 %
sodium ascorbate	0,03 %
pepper	0,09 %
nutmeg	0,07 %

A mixture of two 3-day old sausages containing atypical streptobacteria was used as starter. Each meat plant supplied one of the sausages.

Table 2 : Effect of dry sausage ripening on the protein breakdown pattern

Mean Molecular weight (1)	Name protein	increase (+) or decrease (-) in percentage (2)
198.200	Myosin	- 48,8 %
174.300	M-protein	+
151.500	C-protein	+
145.400		+ 100 %
137.500		+ 100 %
131.900		+ 100 %
100.300	α-actinin	+
93.700		+
80.500		+
75.000		+
60.200		+
53.400	Desmin	+
44.800		+
42.300	Actin	- 32,7 %
39.200	Troponin T	- 26,5 %
35.700	Tropomyosin	+ 75,9 %
33.200		
29.900	30.000 D	
28.100		
25.000	Myosin light chain 1	
21.900	Troponin I	
19.200	Troponin C + myosin light chain 2	
13.900	Myosin light chain 3	
13.300		+
11.300		+
9.700		+

(1) mean of 28 replicates
(2) if the relative increase or decrease is smaller than 10 % only a + (increase) or - (decrease) sign is noted.

Figure 1 : PAGE (8% T, 3% C) of a dry sausage ripening

(A= day of filling, B= day 21)