Study of proteolysis during the curing of dry sausages manufactured with good quality pork.

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INTRODUCTION.

The origin of proteolysis has been throughout the years a subject of studies and a source of controversy particulary regarding microbiological and/or muscle tissue influences. So, CANTONI et al (1975) impute to Micrococci a great proteolytic and peptidasic activity and a decarboxilasic activity to Lactobacilli, but MARTIN (1975) previously painted a higher activity to Lactobacilli making them responsible of the aminoacids appearance. PETAJA (1977) state that proteolysis is owing to the action of Gram negative bacteria.

The muscles enzymes action seem to increase when the dry sausage pH approaches to the optimum pH of catheptic enzymes (pH 6,5) so his activity will depend of several factors like animal race, breeding, feeding, etc. (FOURNAUD, 1976).

Consistently, the dry sausage pH will be determined by the concentration of acid compounds (organic acids) and basic compounds (basic aminoacids peptides and NH_3 mainly) liberated during dry sausage ripening by the action of tissue enzymes as well as of bacterial enzymes.

The proteolysis produce an increase of soluble amínic and amoniacal nitrogen during the firsts days of ripening. Several parameters have been suggested as being indicative of rpoteolysis and so the soluble non protein nitrogen/totals nitrogen has been defined as a possible pointer to the extent of proteolysis (AMBANELLI et al. 1968).

In the present work the relationship between microbial evolution and nitrogenous fractions in dry sausages manufactured from good quality pork was studied to determine the microbial influence in the proteolysis during fermentation time and drying.

EXPERIMENTAL METHODS.

The formulation of dry sausages was: shoulder pork and fresh pork backfat in a proportion of 75 to 25, salt 27 g/Kg, potassium nitrate 0,51 g/Kg, sucrose 5 g/Kg and black pepper 2,73 g/Kg.

Fermentation ocurred for 72 hours at 18°C and at a relative humidity of 75-95%.

Drying was performed for approximately 40 days at 12-15°C and 65-90% of relative humidity.

Samples to be analyzed microbiologically and physical-chemically were taken at different times during the process (stuffing, during fermentation and drying). Methods, media and incubation conditions used for microbiological analyses are presented in Table 1.

The physical-chemical parameters analyzed were: a) The pH determination was done with a postable CRISON pH meter with a penetration electrode. b) Total nitrogen. The employed method was the oficial Kjeldahl (B.O.E. nº 207.29/8/1979). c) Soluble nitrogen. The solubilization of the sample was done at 4°C overnight. After centrifuging, a portion of the supernatant was determined by Kjeldahl. d) Soluble non protein nitrogen. Equal amounts of tricloracetic acid 10% were added to the former supernatant. Macromolecule precipitation was done overnight at 4°C, followed by the supernatant determination by Kjeldahl. e) Ammoniacal nitrogen. The sample was left overnight in water at 4°C. After centifuging we distilled a supernatant portion adding 25 ml of CO_3Na_2 10%. f) Electrophoretic study. After degreasing and drying the sample, the proteins were solubilized and desnaturalized (CALSINA et al. 1982) the PAGE-SDS method was used with a three hours run. The protein bands were dyed with Comassie blue and the densitograms were performed with a VERNON 31 densitometer.

RESULTS AND CONCLUSIONS

We observed a decrease in pH during the firsts days, mainly as a result of microbial fermentation . The weight losses at the end of drying were great as expected for this type of product, wich is subject to progressive dehydratation (see Figure 2). The more representative microbial groups and the physical-chemical parameters related to proteolysis during fermentation and drying of dry sausage are shown in Figure 1 (a-h) a)the amoniacal nitrogen and the evolution of Lactobacilli mainly in the firsts steps of process are very similar. g)there is a clear parallelism between the proteolytic microorganisms and the soluble non protein nitrogen. According to the other microbial groups represented it seems to exist no correlation with the nitrogenous fractions. From these results we could point that the bacterial groups with great responsability in the proteolysis ocurring throughout the ripening are Lactobacilli and proteolytic microorganisms, without discarting the contribution of catheptic enzymes althoug these probably operate during the firsts days of the process when the pH is nearer to the optimum (pH 6,5). This hypothesis agree with the fact that the soluble non protein fraction (aminoacids mainly and small peptides) increases since the first day of manufacture (stuffing).The proteolytic microorganisms could exercise a peptidasic and proteolytic activity whereas the Lactobacilli could degrade the aminoacids by his decarboxilases (CANTONI et al. 1975) facilatating the desaminasic action of Micrococcaceae to NH_3 production. In this study this bacterial group keeps his practically constant throughout the ripening of fermented sausage however we couldn't refect his metabolizing function. An electrophoregram (method PAGE-SDS) was performed in order to visualize the proteolysis along the ripening of dry sausage. A loss of definition was observed (see Figure 3) in the protein bands throughout the drying process, since the proteolysis increases the aminoacidic remainders of proteins decreased and for this reason the molecular weights variety rise. The densitograms related to the following samples are shown in figure 4: A (zero or stuffing), B (72 hours or the end of fermentation) and C (45 days or the end of drying). The differences observed between stuffing and the end of fermentation were small, only a short increase in the peaks corresponding to low molecular weight bands (14 K), however the differences between the end of fermentation and the end of drying, and also in the low molecular weights (from 46 K to 14 K) were greater. A loss of definition in the peaks corresponding to the protein bands following actine (46 K) was observed due to the proteolysis throughout drying in agreement with the fact that this proteins 43,5 K, 39 K, 35,5 K are citoplasmatic proteins so more accesible to the enzymatic attack. The slight definition of densitogram C, mainly in the low molecular weights zone, could be imputed to proteic remainders as result of proteolysis.

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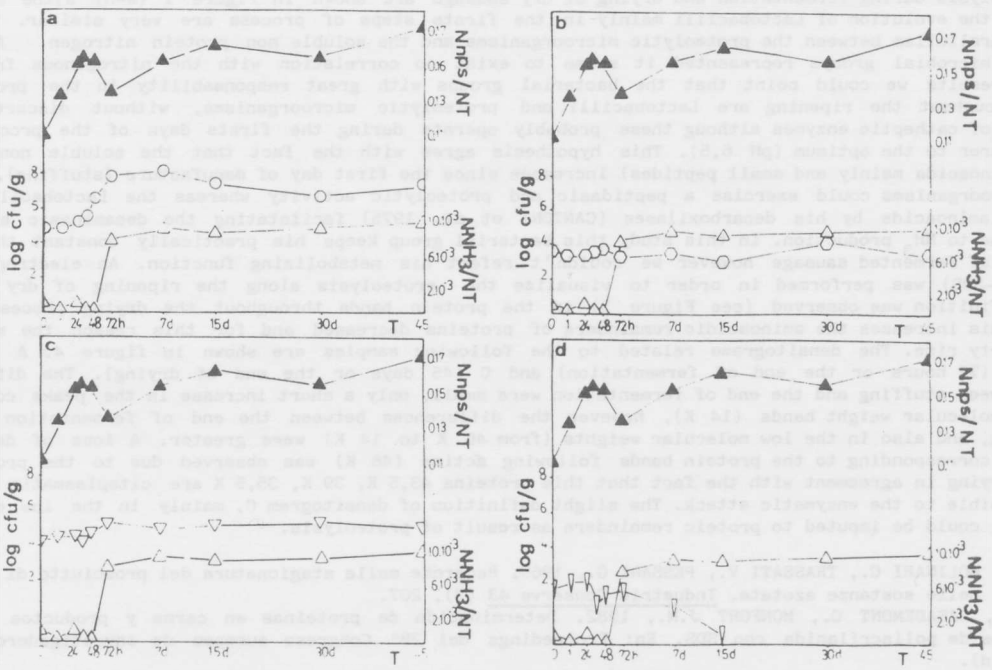
TABLE 1. Methods, media and incubation conditions used for microbiological analyses.

Microorganisms	Method	Media	Incubation	
Aerobic total count	Plating	P.C.A. (Oxoid)	30°C	72 h.
Lactobacilli	plating	M.R.S. agar (Merck)	37°C	72 h.
Proteolytics	Spreader	Calcium caseinat agar (Merck)	30°C	72 h.
Enterobacteriaceae	plating	U.R.B.G. agar (Oxoid)	30°C	24 h.
Enterococci	spreader	Slanetz and Bartley medium (Oxoid)	37°C	24-48 h.
Yeasts and fungi	plating	Sabourand dextrose 2% agar (Merck)	22°C	5 days
Micrococcaceae	spreader	Manitol-salt phenol-red agar (Merck)	22°C	72 h.
B. thermosphacta	spreader	GARDNER (1966)	22°C	72 h.
V. costicola and haloto-lerant Gram negatives	spreader	C.U.K.A. (GARDNER, 1973)	22°C	72 h.

FIGURE 1. Evolution of the physical-chemical parameters related to proteolysis (▲) Nnps/NT (Soluble non-protein Nitrogen/Total Nitrogen) and (△) N-NH_3 /NT (Amoniacal Nitrogen Total Nitrogen) and the microbial evolution during dry sausage ripening: a) Lactobacilli, b) Enterococci, c) Micrococcaceae, d) Enterobacteriaceae.



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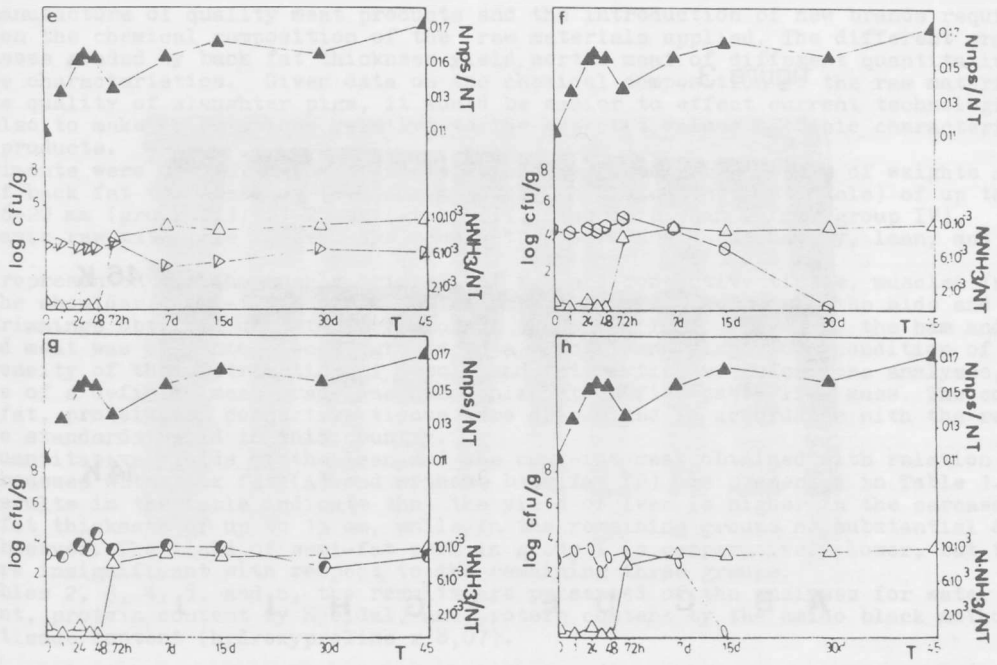

FIGURE 2. Evolution of some physical-chemical parameters during dry sausage ripening.
a) Nitrogenous fractions: (■) Soluble Nitrogen/Total Nitrogen; (▲) Soluble non-protein Nitrogen/Total Nitrogen; (△) Amoniacal Nitrogen/Total Nitrogen.
b) Weight losses percentage.
c) pH evolution.

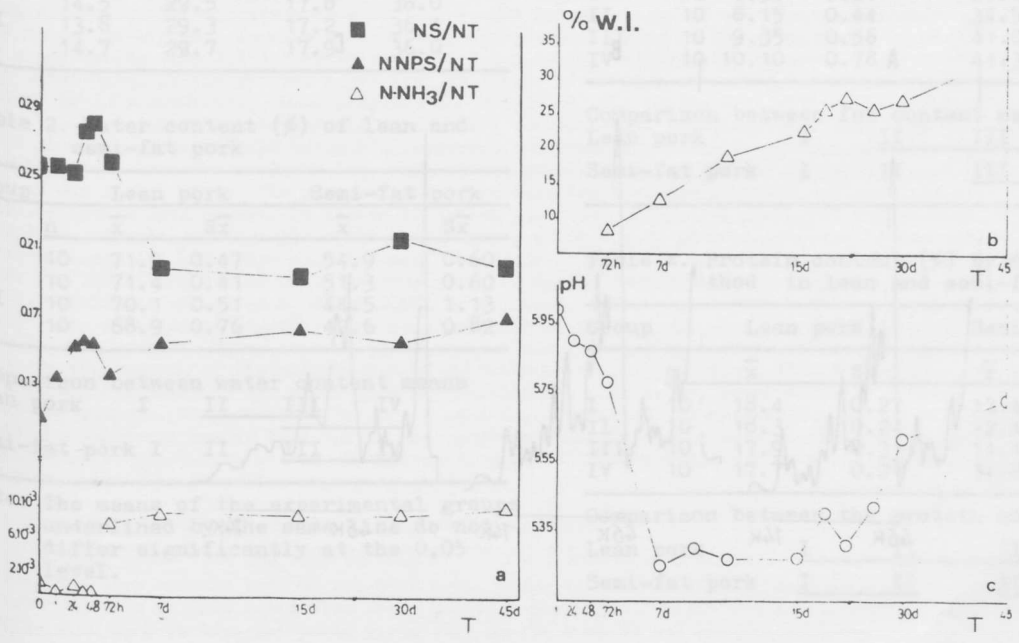

FIGURE 3. Electrophoregram (SDS-PAGE method) of the following samples: A (Stuffing), B (24 hours of temperature equilibration), C (24 h. of fermentation), D (36 h. of fermentation), E (48 h. of fermentation), F (72 h. of fermentation), G (7 days of ripening), H (15 d. of ripening), I (30 d. of ripening), J (45 d. of ripening).

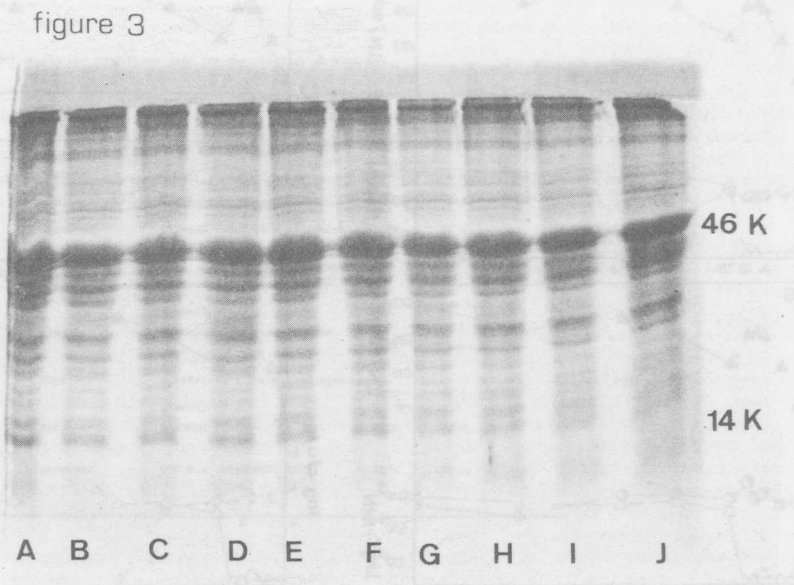

FIGURE 4. Densitograms corresponding to three different points of the dry sausage ripening process A) Stuffing, B) At the end of the fermentation, C) At the end of the drying.