PROTEINASE ACTIVITIES IN SPANISH DRY-CURED HAM MANUFACTURED WITH MEAT OF DIFFERENT QUALITY.

GIL, M., ARNAU, J. AND SARRAGA, C.

IRTA. Centre de Tecnologia de la Carn Granja Camps i Armet, s/n 17121 Monells (Girona) Spain.

INTRODUCTION

The interest in the identification and characterization of proteinases in different conditions of muscle and meat has clearly increased during last years.
Demand for quality cured ham has stimulated research on the commercial and technical aspects of this product as well as the biochemical processes that take place in the muscle from the animal´s deat .h to the final elaborated product.

Quality of the final product is influenced by some factors as the rate of penetration of the salt, the distribution of the curing agents on the muscles, and the water holding capacity (WHC). These factors are directly related to meat quality. Hams elaborated with PSE meat present important drip and protein losses since the WHC of PSE meat is lower than the WHC of normal meat. Penetration of salt is accelerated. In DFD hams, on the contrary, penetration of salt is restrained and putrefying bacteria growth enhanced due to the high pH values of DFD meat. In both cases, low quality products are obtained (Arnau, 1988).

Proteolytic enzymes have been found to be responsible for meat tenderization, (Etherington, 1984; Goll et al., 1983; Kay, 1982), and studies on their role in the curing process of Spanish ham are on the way. Proteolytic activities of calpains and cathepsin D are influenced by curing agents (Sárraga et al., in press).

The aim of this work is to determine the effect of meat quality on the proteolytic activity of hams during the Spanish dry-curing process.

MATERIALS AND METHODS

MANUFACTURING TECHNOLOGY

The manufacturing process of Spanish cured ham is very different from those of other countries and even depends on the area of Spain where it is carried out. The technology used in this study is summarized on the following table:

Step	Temperature (ºC)	Time (days)
Salting	3-5	20
Salt-equalization	3-5	30
First step of ripening	6-8	15
	8-10	15
	12-14	15
	16-18	25
End of ripening	20-22	20
	22-24	30
	28-30	32

Selection of fresh ham was done by measuring pH, electric conductivity (Quality Meater) and light scattering (fiber optic probe).

Samples were obtained after the following steps: salting, salt-equalization, first step of ripening and end of ripening. Fresh muscle samples were taken as controls. Semimembranosus SM, (external) and Biceps femoris BF,(internal) muscles of three hams of each quality were used in each step.

PREPARATION OF MUSCLE EXTRACTS

Muscle samples were trimmed of fat and connective tissue. They were homogenized in an Ultra-Turrax(15.000rpm 20 s.) at 0-4ºC in the appropriate cold buffer. The homogenats were centrifuged at 20.000 x g, 20 min. at 4ºC and supernatants were filtered and used as enzymatic sources.

ENZYMATIC ACTIVITY ASSAYS

CALCIUM-DEPENDENT PROTEASE (CALPAIN)
Samples were homogenized (1/20, w/v) in 50 mM Tris-HCl,pH 7.4. The activity was measured according to Waxman and Krebs' method (1978).The 200 µl reaction mixture contained: 100 µl of

enzyme sample, 5 mg/ml α-casein(Sigma Chemical Co.) 50 mM Tris-HCl (7.4) 3 mM 2-mercapto-ethanol and 5mM $CaCl_2$. The calcium was added last to initiate reaction, which was allowed to proceed overnight at 37ºC. 160 µl of ice cold 10% trichloroacetic acid (TCA) was added and the mixture left on ice for 1 h. The precipitate protein was removed by centrifugation at 4.000 rpm, 10 min, and the absorbance of the supernatant was read at 280 nm. Controls were run that contained 1 mM EDTA instead of calcium.

CATHEPSIN D

Homogenization of the samples (1/3.5, w/v) was carried out in 250 mM sodium formate buffer, pH 3. The activity was determined by the method of Anson (1938), using hemoglobin as substrate. The 2,5 ml reaction mixture contained 100 µl of enzyme sample, 25 mg of hemoglobin, 250 mM sodium formate buffer, pH 3. After incubation at 37ºC for 6 h the reaction was stopped with ice cold 10% TCA and the mixture filtered through Whatman nº 50 paper. Absorbance at 280 nm was monitored. Controls were run by adding TCA before the enzyme sample.

One enzyme unit was defined as that amount which caused a change in absorbance of 0.001 units per min. Specific activity was defined as enzyme units per mg protein.

PROTEIN DETERMINATION

Protein concentration was determined by the method of Lowry et al.(1951), using bovine serum albumin as standard.

RESULTS

Figure 1 shows the evolution of calpains specific activity during the elaboration process.

Calpains activity at the end of the process showed similar values to those of fresh muscle. A significant increase of activity was found after the salt-equalization step. Activity in BF muscle was higher than in SM muscle, whereas differences depending on meat quality were observed.

The enzyme activity was lower in DFD hams than in normal hams. On the contrary, PSE hams showed higher calpain activity than normal hams. In both cases, differences of activity on this step were statistically significative according to the test of Mann-Whitney (comparisons significant at the 0.01 level).

Figure 2 shows the evolution of cathepsin D activity during the manufacturing process. No relevant changes in the proteolytic activity were found by comparison between fresh muscle and hams at the end of ripening. BF muscle presented more activity than SM muscle during the whole process. There was an increase on the activity after the salting step in normal hams. This increase was lower in PSE hams and was not detected in DFD hams.

CONCLUSIONS

1. During the initial steps of the ham curing process, external muscles (SM) get higher NaCl concentration than internal ones(BF). High concentration of NaCl was found to cause the inhibition of calpain and cathepsin D activities (Sárraga et al., in press), so that it could explain the lower proteolytic activity found in SM muscle with respect to BF muscle.

2. Proteolytic activity increases during the first steps of the curing process in PSE and normal hams, and tend to get similar values irrespective of meat quality as the process goes on and activity decreases.

DFD hams show lower activities than PSE and N hams during the whole process.

3. After the salt-equalization step, PSE hams show higher calpain activity than normal hams. This increase of activity could be justified by the elevated levels of sarcoplasmic Calcium described by Cheah et al.(1984) in PSE meat.

REFERENCES

-Anson ML (1938):
The stimation of pepsin, trypsin, papain and cathepsin with haemoglobin.

J. Gen. Physiol, 22, 79.
-Arnau J. (1988)
Defectos en el jamón curado. In "El Jamón Curado. Aspectos técnicos" (Arnau J, Hugas M, Monfort JM, eds.) Girona, 189-200.
-Cheah K S, Cheah A M, Crosland A R, Casey J C.(1984):Relationship between Calcium release, sarcoplasmic Calcium, glycolysis and meat quality in halotane-sensitive and halothane-insensitive pigs.
MeatSci. 10, 117-130.
-Etherington D J (1984):
The contribution of proteolytic-enzymes to post mortem changes in muscle.
J. Animal Sci. 59(6), 1644-1650.
-Goll D E, Otsuka Y, Nagainis P A, Shannon, J D, Sathe SK, Muguruma M (1983):
Role of muscle proteinases in maintenance of muscle integrity and mass.
J.Food Biochem. 7, 137-177.
-Kay J (1982):
Proteolysis- a degrading business but food for thought.
Biochem. Soc. Trans. 10 (4), 277-279.
-Lowry O H, Rosenbrough N J, Farr A L, Randall R J (1951): Protein measurement with Folin-Phenol reagent.
J. Biol. Chem. 193, 265-275.
-Sárraga C, Gil M, Arnau J, Monfort J M, Cussó R: Effect of curing salt and phosphate on the activity of porcine muscle proteases. Meat Sci. (in press).
-Waxman L, Krebs E G (1978):
Identification of two protease-inhibitors from bovine cardiac muscle.
J. Biol. Chem. 253, 5888-5891.

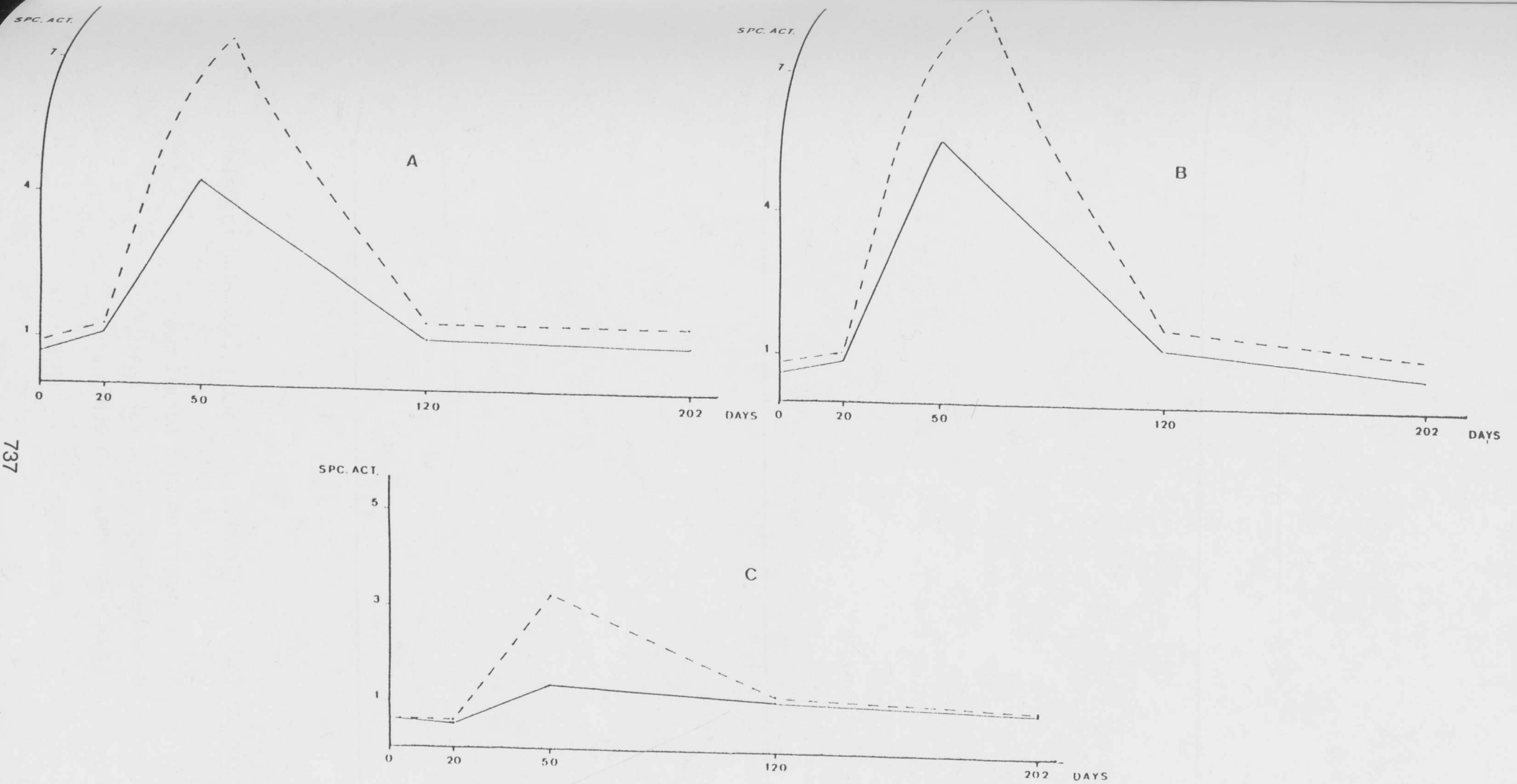

Figure 1. Evolution of Calpains specific activity during the elaboration process of Spanish ham.

A) Hams elaborated with Normal meat.

B) Hams elaborated with PSE meat.

C) Hams elaborated with DFD meat.

(_____) SM muscle ; (-----) BF muscle.

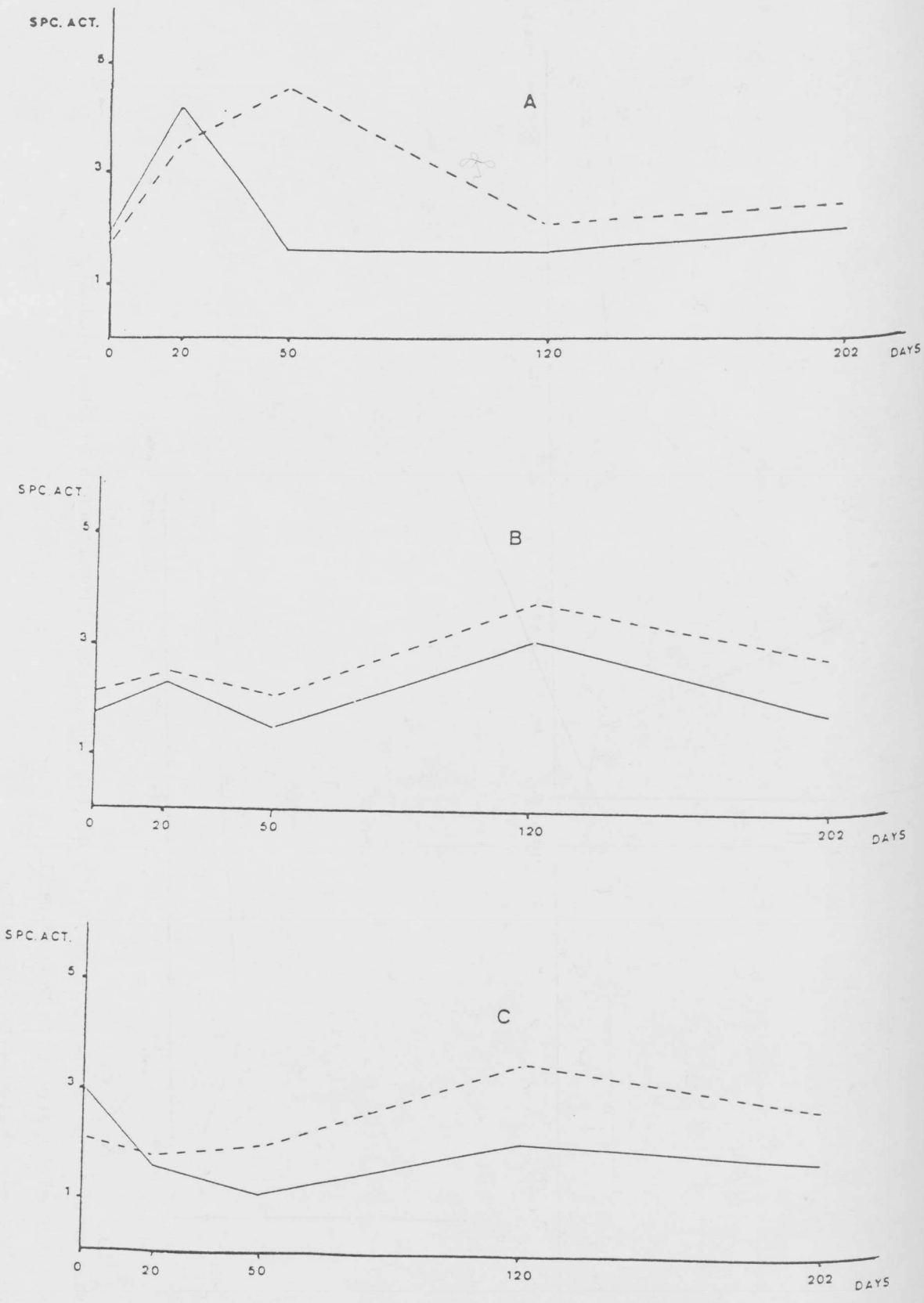

Figure 2. Evolution of cathepsin D activity during the elaboration process of Spanish ham.

A) Hams elaborated with Normal meat.

B) Hams elaborated with PSE meat.

C) Hams elaborated with DFD meat.

(___) SM muscle; (----) BF muscle.

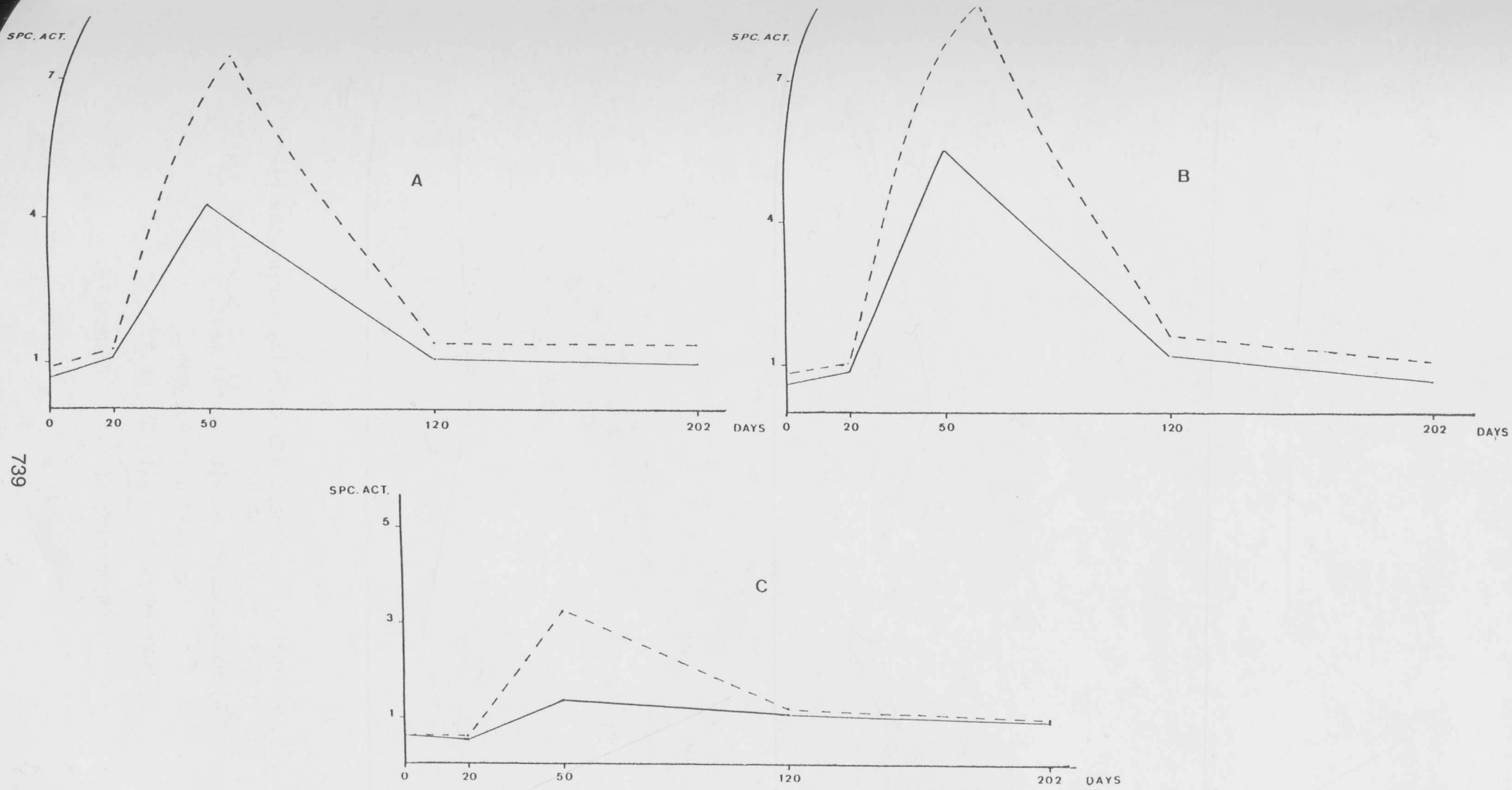

Figure 1. Evolution of Calpains specific activity during the elaboration process of Spanish ham.

A) Hams elaborated with Normal meat.
B) Hams elaborated with PSE meat.
C) Hams elaborated with DFD meat.

(_____) SM muscle; (-----) BF muscle.

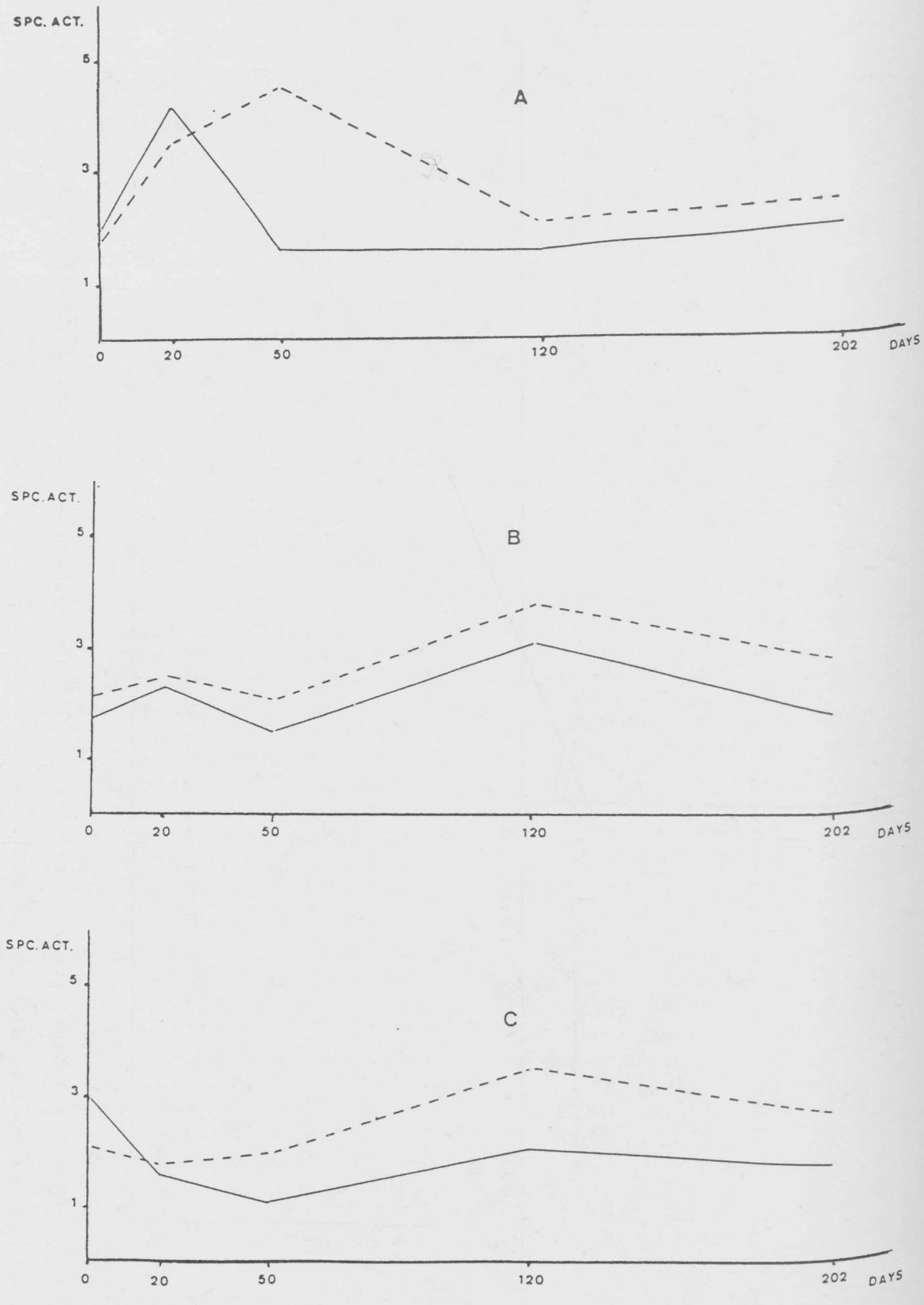

Figure 2. Evolution of cathepsin D activity during the elaboration process of Spanish ham.

A) Hams elaborated with Normal meat.

B) Hams elaborated with PSE meat.

C) Hams elaborated with DFD meat.

(_____) SM muscle; (------) BF muscle.