ENZYME ACTIVITIES IN THE PROCESSING OF DRY-CURED HAM

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SUMMARY

Proteolytic, lipolytic and glycolytic enzymes from muscle and adipose tissue have been assayed for activity at different key points (raw, post-salting, mid-curing and end-curing) in a standard process for dry-cured ham production. The recovered activities indicate that these enzymes are, in general, quite stable even after 7 months of dry-curing. Thus, these recovered activities were in the range 35-45% for cathepsins B, H and L, 60-80% for glycosidases, 40-50% for lysosomal acid lipase and neutral lipase, 30-50% for arginyl and tyrosyl hydrolyzing activities while remained very similar to the initial activity in the case of the basic lipolytic and leucyl hydrolyzing activities. All these enzymes appear to be active along the complete process. In the case of adipose tissue, however, the neutral and basic lipolytic activities almost disappear after 5 months of process. These enzymes are less stable than muscle enzymes and their participation in the biochemical changes is expected to be restricted to the first stages of the process.

INTRODUCTION

Dry-cured ham is a meat product which requires a long processing time. Many biochemical changes take place during the process, mainly of proteolytic and lipolytic nature. Thus, intense proteolysis and lipolysis have been reported for Spanish Serrano ham (Flores et al.,1984, 1985; Aristoy and Toldrá,1991; Toldrá et al.,1991 a), Italian Parma ham (Bellatti et al.,1985) and American Country-Style ham (Mc Cain et al.,1968). Substantial activities of muscle proteinases and glycosidases have been recovered after 8 months of dry-curing (Toldrá and Etherington,1988). Futhermore, these enzymes have been found quite active when assayed "in vitro" in the presence of curing agentes and process parameters typical of that process (Rico et al.,1990, 1991 a; Toldrá et al.,1991 b). Adipose tissue and muscle lipases and esterases have been also recently assayed (Motilva et al., 1991 a, b). All these enzymes may have an important role in the dry-curing process although it is not well known, yet.

The objective of this study is the assay of activity of the main muscle and adipose tissue enzymes (cathepsins B,H,L,D, aminopeptidases, glycosidases, lipases and esterases) at different stages in the processing of dry-cured ham.

MATERIALS AND METHODS

Preparation of enzymes extracts: Samples of M.Biceps femoris and adipose tissue were removed at different stages of the dry-curing process: Raw (time 0), after post-salting (2 months) and three stages of drying (3.5, 5 and 7 months). Three hams were assayed at each stage. The enzyme extracts were prepared as previously described (Toldrá and Etherington,1988; Rico et al.,1991 b,c; Toldrá et al.,1991b; Motilva et al.,1991).

Assay of the enzymes activities: The assayed enzymes are lysosomal proteinases (cathepsins B,D,H and L), aminopeptidases (Leucyl, arginyl and tyrosyl hydrolyzing activities), glycosidases (B-glucosaminidase and N-acetyl-B-glucosaminidase) and lipases (acid esterase and acid and neutral/basic lipase activities in muscle and neutral/basic lipase and acid esterase activity in adipose tissue). Cathepsin D was assayed at 45ºC and pH 3.5 by using haemoglobin as substrate (Rico et al.,1991 b). The rest of enzymes were assayed at 37ºC by using the respective 4-methylumbelliferyl and 7-(4-methyl)coumarylamide substrates (Sigma). Four samples and controls were measured for each enzyme and experimental point.

Chemical analysis: Protein and fat content in muscle and adipose tissue were determined by Official Standard methods based on Kjeldahl semimicro-method (Presidencia del Gobierno,1979) for protein and Soxhlet (ISO,1973) for fat content.

RESULTS AND DISCUSSION

The evolution of the residual enzyme activities along the dry-curing process is given in figures 1-4. The recovered activities indicate that these enzymes are, in general, quite stable even after 7 months of

processing. Cathepsins B,H and L remained quite active (around 40% of the initial activities) along the process (see fig. 1). On the other hand, cathepsin D almost disappeared after 5 months although substantial activities have been recovered in previous experiences with other hams (Rico etal.,1991 b,c).

Muscle aminopeptidases showed a good stability (see fig.2). So,leucyl hydrolyzing activity was very similar to that in raw ham while arginyl and tyrosyl hydrolyzing activities were recovered in a 59 and 33%, respectively. B-glucuronidase and N-acetyl-B-glucosaminidase (see fig.2) were also very stable (86 and 55% of recovered activity, respectively).

Muscle lipases showed better stability than those from adipose tissue (fig.3 vs fig.4) which almost disappeared after 5 months of process. Muscle basic lipolytic activity was very similar to the initial activity in raw ham. However, neutral and lysosomal acid lipase activities were recovered in a similar percentage as cathepsins B,H and L, around 40% (see fig.3). The recovery of esterase activity in both muscle and adipose tissue were around 55 to 60% (see fig.3 and 4).

In general, it can be concluded that most of the assayed enzymes are quite stable, with substantial activity recoveries even after 7 months of processing. The mechanism responsible of this extreme stability along the dry-curing process still remains unclear. It has been suggested that curing salts might be stabilizing the enzymes (Toldrá and Etherington,1988) and further research is being carried out on this hypothesis. These enzymes may have an important participation in the biochemical changes (proteolysis and lipolysis) along the dry-curing process.

CONCLUSIONS

The results indicate that muscle enzymes (cathepsins B,H,L, glycosidases, aminopeptidases, lipases and esterases) and esterases from adipose tissue are quite stable even after 7 months of dry-curing process. Lipases from adipose tissue are active only in the first 5 months. The mechanims for this stability are still unknown although it could be due to the curing salts. This hypothesis is being investigated.

Figure 1.- Evolution of the activities of cathepsin B (+), B+L (•), H (*) and D (•) along the processing of dry cured ham.

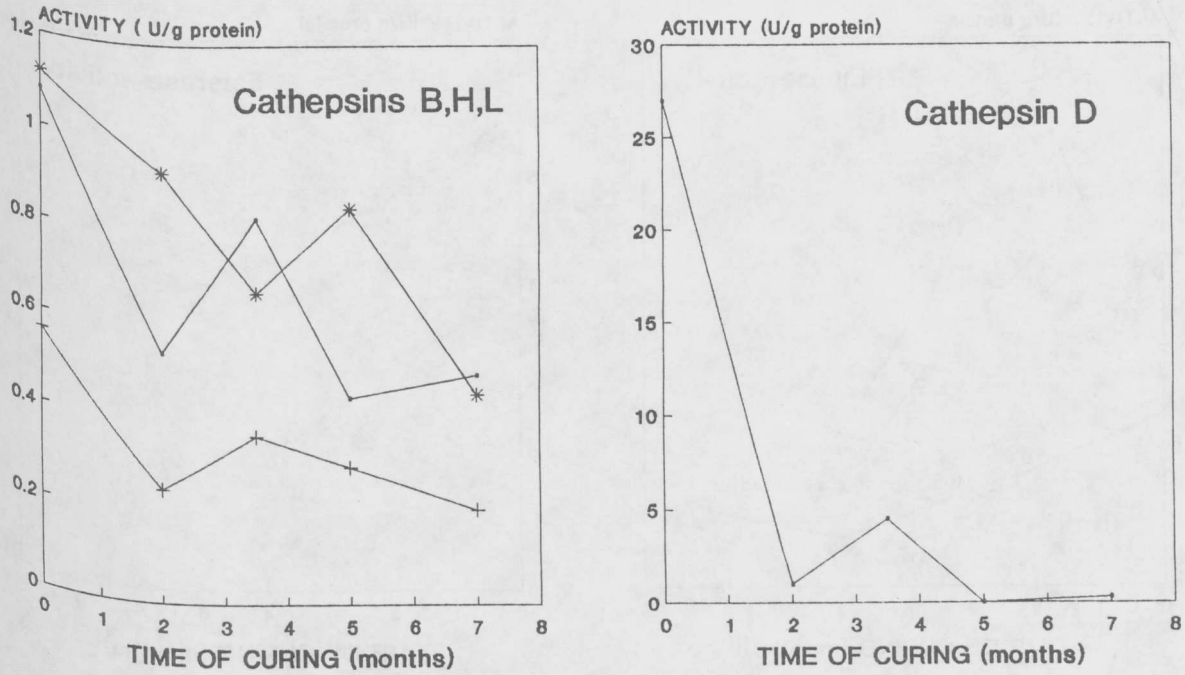


Figure 2.- Evolution of the Leucyl (•), Arginyl (+) and Tyrosyl (*) hydrolyzing activities, and B-glucuronidase (•) and N-acetyl-B-glucosaminidase (+) along the processing of dry-cured ham.

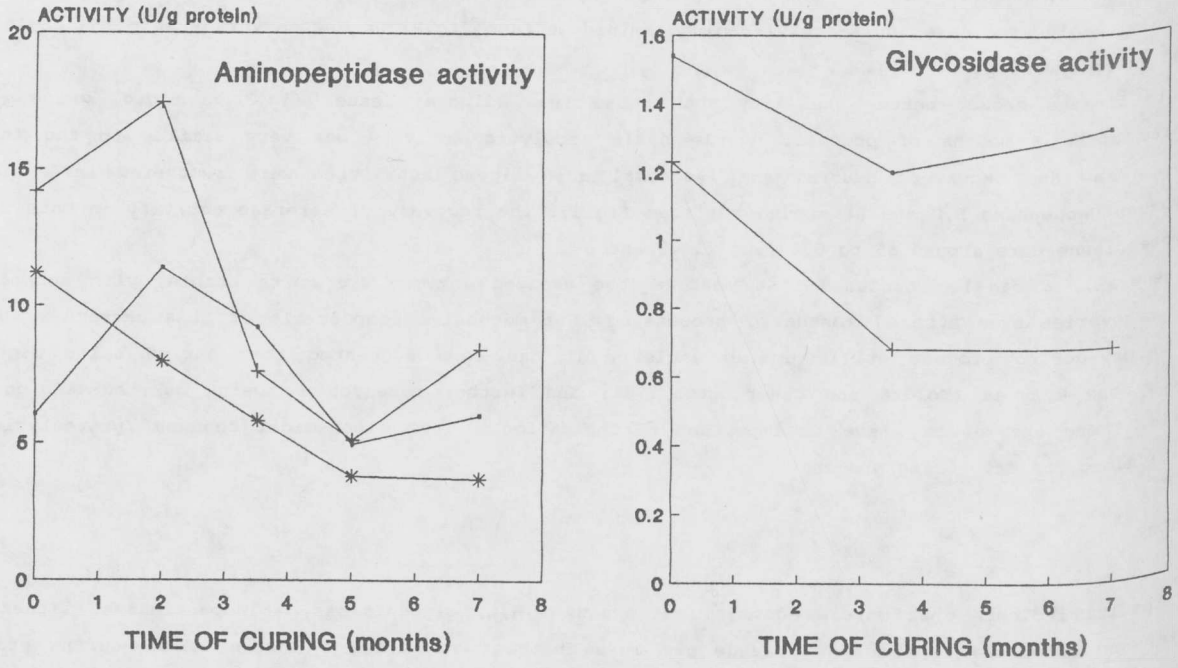


Figure 3.- Evolution of the activities of muscle lipases,pH 5.0 (•), pH 7.0 (+), pH 8.5 (*) and esterase (•) along the processing of dry-cured ham.

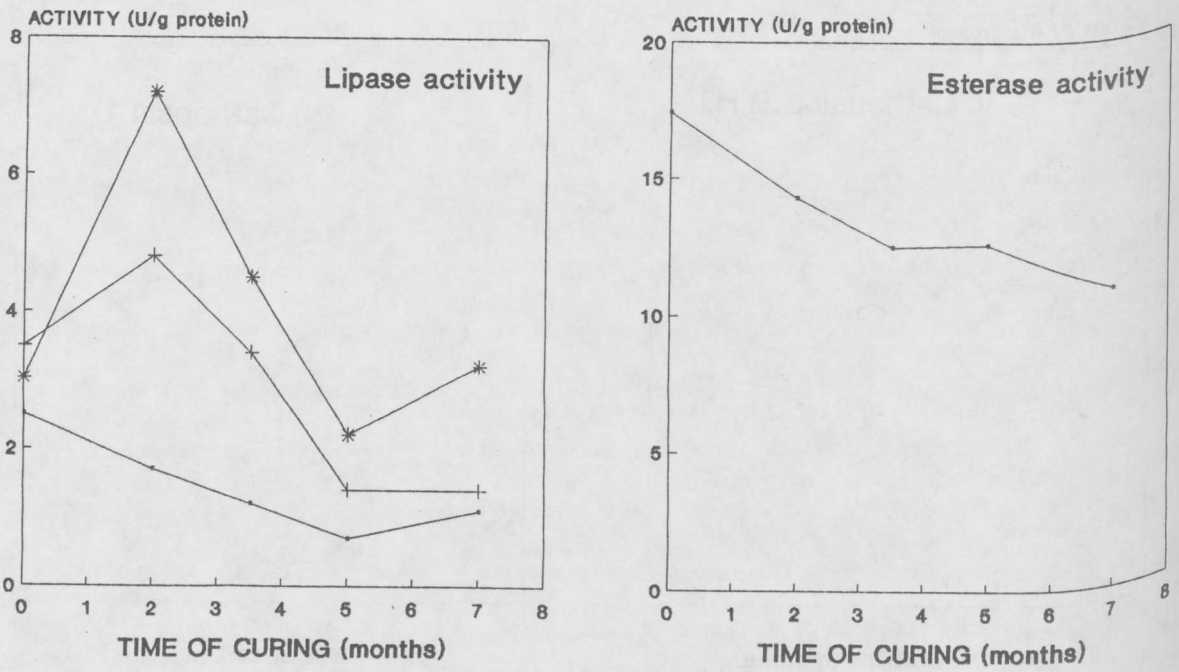
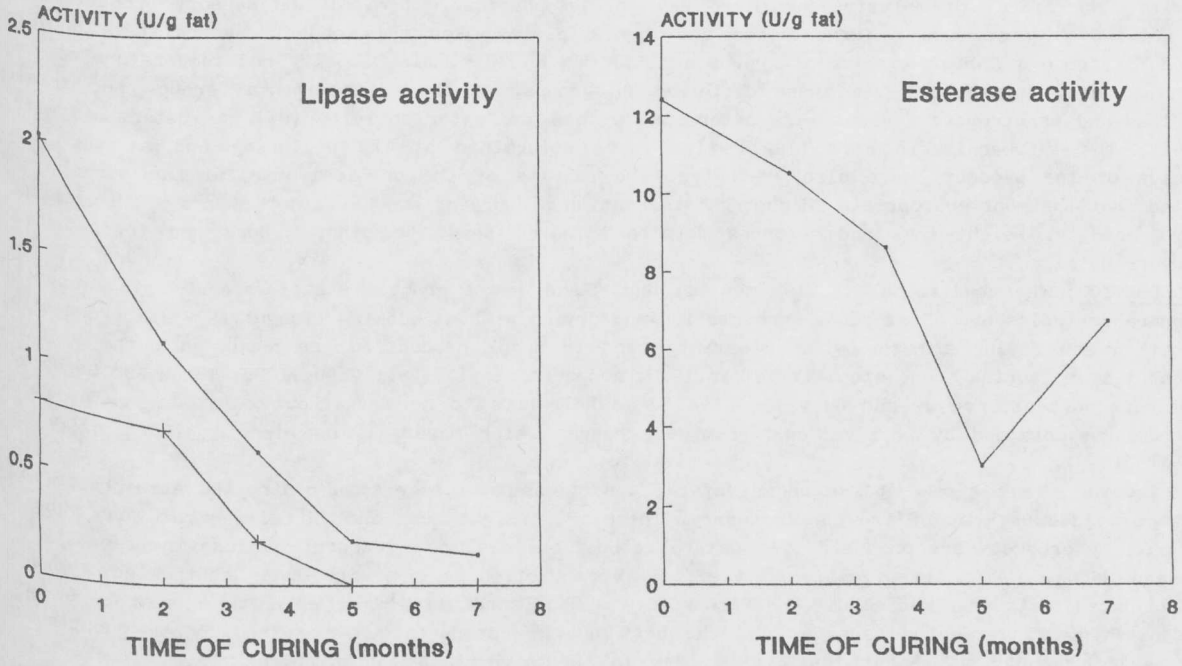


Figure 4.- Evolution of the activities of adipose tissue lipases, pH 7.0 (•), pH 8.5 (+) and esterase (•) along the processing of dry-cured ham.



REFERENCES

ARISTOY, M-C.; and TOLDRA, F. (1991) "Deproteinization technique for HPLC amino acids analysis in fresh pork muscle and dry-cured ham". J.Agric.Food Chem., submitted.

BELATTI, M.; DAZZI, G.; CHIZZOLINI, R.; PALMIA, F. and PAROLARI, G. (1985) "Modifications chimiques et physiques des proteines an cours de la maturation du Jambon de Parme". V.P.C.6, 142-145.

FLORES, J.; BERMELL, S.; NIETO, P. and COSTELL, E. (1984) "Cambios químicos en las proteinas del jamón durante los procesos de curado, lento y rápido, y su relación con la calidad". Rev. Agroquim. Tecnol. Aliment. 24,503-509.

FLORES, J.; NIETO, P.; BERMELL, S. and MIRALLES, M-C. (1985) "Cambios en los lípidos del jamón durante el proceso de curado lento y rápido, y su relación con la calidad". Rev. Agroquim. Tecnol. Aliment. 25, 117-124.

ISO (1973) Meat and meat products. determination of free fat content. 1444. Int. Org. Standard.

McCAIN, C. R.; BLUMER, T.R.; CRAIG, H.B. and STEEL, R.G. (1968) J.- Food. Sci. 33, 142-145.

MOTILVA, M-J., TOLDRA, F. and FLORES, J.(1991) "Assay of lipase and esterase activities in fresh pork meat and dry-cured ham" Meat Sci., submitted.

RICO, E.; TOLDRA, F. and FLORES, J. (1990) "Activity of cathepsin D as affected by chemical and physical dry-curing parameters" Z. Lebensm. Unters. Forsch. 191, 20-23.

RICO, E.; TOLDRA, F. and FLORES, J. (1991a) "Effect of dry-curing process parameters on pork muscle cathepsins B, H and L activities" Z. Lebensm. Unters. Forsch. Accepted for publication.

RICO, E.; TOLDRA, F. and FLORES, J: (1991b) "Assay of cathepsin D activity in fresh pork muscle and dry-cured ham" Meat Sci. 29, 287-293.

RICO, E.; TOLDRA, F. and FLORES, J. (1991c) " Problems associated with the assay of cathepsin D in meat and meat products" Food Chem., 40, 87-91.

TOLDRA, F. and ETHERINGTON, D.J. (1988) "Examination of cathepsins B, D, H and L activities in dry-cured hams" Meat Sci. 23, 1-7.

TOLDRA, F.; MIRALLES, M-C. and FLORES ,J. (1991a) "Protein extractability in dry-cured ham" Food Chem. submitted.

TOLDRA, F.; RICO, E. and FLORES, J. (1991b) "Activities of pork muscle proteases in cured meats" Biochimie, submitted.