ELSEVIER

Meat Science, Vol. 46, No. 4, 379–385, 1997

PII: S0309-1740(97)00032-6 0309-1740/97 $17.00+0.00

Changes in Proteins During the Ripening of Spanish Dried Beef 'Cecina'

I. García, V. Díez & J. M. Zumalacárregui

Departamento de Higiene y Tecnología de los Alimentos, Facultad de Veterinaria, Universidad de León, 24071 León, Spain

(Received 31 January 1997; revised 13 March 1997; accepted 14 March 1997)

ABSTRACT

Changes in the solubility of sarcoplasmic and myofibrillar proteins were tracked in Semitendinosus *and* Rectus femoris *muscles during the ripening process of Spanish 'Cecina'. The extractability of both types of proteins decreased during the ripening. This phenomenon was more marked in the initial stages of processing. Electrophoretic studies of the myofibrillar proteins showed the virtual disappearance of the myosin heavy chain, troponin C and myosin light chain 2 from the smoking phase onward and the appearance of three components of molecular weight of about 65, 70 and 75 kda during ripening. The remaining proteins did not suffer appreciable changes.* © *1997 Elsevier Science Ltd*

INTRODUCTION

The origin of meat curing is lost in antiquity. Its first use was for preserving meats during times of abundance against times of scarcity. Today, cured meats are attractive to consumers as they represent a great variety of products; their characteristic colour and flavour depending on local customs and habits.

Traditional intermediate moisture foods (IMFs) made from whole joints of pork and beef are highly acceptable in different parts of the world. Spanish 'cecina' is a salted, dried and smoked meat product manufactured almost exclusively in the province of León (Northwestern Spain). 'Cecina' resembles South American 'charqui' and 'carne seca' and European 'bündnerfleisch' and 'bresaola' (García *et al.*, 1995).

Cured products manufactured from beef are particularly rich in protein. The final content in products with a humidity of 45–50% is about 35% (Souci *et al.*, 1974; Sinell and Hentschel, 1977; Gutiérrez *et al.*, 1988; Kotzekidou and Lazarides, 1991).

During processing of these meat products, the proteins undergo various modifications which depend on numerous parameters. These changes take place in two clearly differentiated stages: before and after salting.

Studies of the changes undergone by proteins during the conversion of muscle to meat have focused on understanding the mechanism of meat tenderisation. The protein changes

taking place during ripening of dry-cured hams and dry-fermented sausages have been studied exhaustively (García de Fernando and Fox, 1991; Toldrá *et al.*, 1992, 1993; Córdoba *et al.*, 1994; etc.). However, despite the fact that modifications in proteins could be an important source of flavour compounds, little scientific information is available in respect of dried beef products.

The aims of this research were to study the extractability of myofibrillar and sarcoplasmic proteins and the electrophoretic changes that occur in myofibrillar proteins during the processing of dry beef cecina.

MATERIALS AND METHODS

Muscle samples

Three pieces of fresh 'babilla' (composed of by *M. Rectus femoris* and *Vastus lateralis*, *V. medialis* and *V. intermedius*) and three of 'contra' (*M. Semitendinosus* and *Gluteobiceps*) with a mean weight of 6·5–7 kg each, were covered with a mixture of coarse salt (*ca.* 0·15 kg kg^{-1}) and sodium nitrite (50–60 ppm) and held at 3–4°C and relative humidity (RH) of 85–90% for 72 hr. They were then washed to remove excess salt and held under the same conditions for another 30 days (post-salting or salt equalisation stage). The meat was then placed in a smoke house at a temperature of 12–15°C, smoke being produced by burning a mixture of oak and beech chippings in the room. The joints were then dried for 40 days at 10–12°C and 75–80% RH in a conditioning room. Finally, the meat was aged in a cellar for several weeks (more than 60 days in a cold, dry environment).

M. Semitendinosus and *Rectus femoris* were analysed. Samples of each muscle type were removed on day 0 (corresponding to the day of salting), after washing (day 3), at the end of salting (day 33), smoking (day 53), and drying (day 93), and at the end of the process (153 days).

Water activity (a_W) was determined at 25°C as described by Serrano Moreno (1979). Moisture, pH and salt content were determined according to Spanish official procedures (Presidencia del Gobierno, 1979).

Protein extraction

Sarcoplasmic and myofibrillar proteins were extracted from 1 g samples as outlined by Helander (1957), using, consecutively, 0·03 M, pH 7·4 phosphate buffer (sarcoplasmic fraction) and 1·1 M IK + 0·1 M phosphate pH 7·4 buffer (myofibrillar fraction).

Determining sarcoplasmic and myofibrillar proteins

The protein concentration was determined by the biuret method (Gornall *et al.*, 1949), using bovine serum albumin (BSA) as the standard.

Myofibrillar proteins SDS–polyacrylamide gel electrophoresis

The protein samples (25 g) were prepared for electrophoresis as described by Davis (1964) and Weber and Osborn (1969), using sodium dodecyl sulphate (SDS). Each sample was mixed in 4 ml of H_2O, 1 ml tris HCL 0·5 M pH 6·8, 0·8 ml glycerol, 1·6 ml SDS 10% (w/v)

0·4 ml 2 β-mercaptoethanol and 0·2 ml bromophenol blue 0·05% (w/v). The mixture was boiled for 4 min before loading samples on to the gels.

SDS–polyacrylamide gel electrophoresis was carried out as described by Laemmli (1970) using 10% polyacrylamide gels. The proteins in the gel were stained with 0·1% Coomassie brilliant blue R_{250} dissolved in methanol/acetic acid/water (40/10/50, vvv) (Penny *et al.*, 1985). Destaining was carried out by diffusion in the fixing solution. Gels were dried by immersing in a solution of 5% glycerol for 30 min and holding at 80°C for 1 hr.

Standard proteins myosin (200 kda), β-galactosidase (116·2 kda), phosphorylase b (92·5 kda), bovine serum albumin (BSA) (66·2 kda) and ovalbumin (45 kda), from Bio-Rad SDS-PAGE were simultaneously run for protein identification. Each gel was scanned at 650 nm with a Shimadzu-LS 930 densitometer.

RESULTS AND DISCUSSION

Table 1 shows the characteristics of cecinas studied.

Protein solubility

Figure 1 shows the changes in the extractability of the sarcoplasmic and myofibrillar proteins of the *Semitendinosus* and *Rectus femoris* muscles in the cecinas.

The extractability of the sarcoplasmic proteins in the *Semitendinosus* muscle diminishes from 289·11 mg to 70·46 mg protein g^{-1} dry matter and in the *Rectus femoris* from 214·76 mg to 46·88 mg protein g^{-1} dry matter. Thus approximately 75% of this type of protein in both muscles has become insoluble. In both muscles the myofibrillar proteins become even more insoluble, extractability decreasing by approximately 82% in both muscles during ripening. The insolubilisation of both types of proteins is more marked during the initial stages of processing.

The drop in extractability of soluble proteins in several types of dry-cured hams has been noted by various authors (Ambanelli *et al.*, 1968; Cantoni and Cattaneo, 1974; Flores *et al.*, 1984; De Prado, 1988; Córdoba *et al.*, 1994). This decrease could be owing to hydrolysis and/or loss of solubility arising from protein denaturation. In Iberian ham it is clear that protein denaturation takes place alongside proteolytic processes, because the observed decrease in solubility is not balanced by an increase in non-protein nitrogen (Córdoba *et al.*, 1994)

TABLE 1
Characteristics of the 'Cecina' Used in the Study[a]

	Raw cecina		*Dry-cured cecina*	
	SM	*Rf*	*SM*	*Rf*
Moisture (%)	74·06 ± 0·06	74·69 ± 0·61	50·31 ± 5·11	32·09 ± 0·95
Water activity	n.d*	n.d*	0·877 ± 0·004	0·834 ± 0·002
pH	5·51 ± 0·00	5·54 ± 0·03	5·88 ± 0·01	5·86 ± 0·01
NaCl (% DM)	n.d*	n.d*	10·24 ± 0·52	8·42 ± 0·23

[a]Means of 6 cecinas ± Standard error.
n.d.* Not determined.
SM = *Semitendinosus*; Rf = *Rectus femoris*.

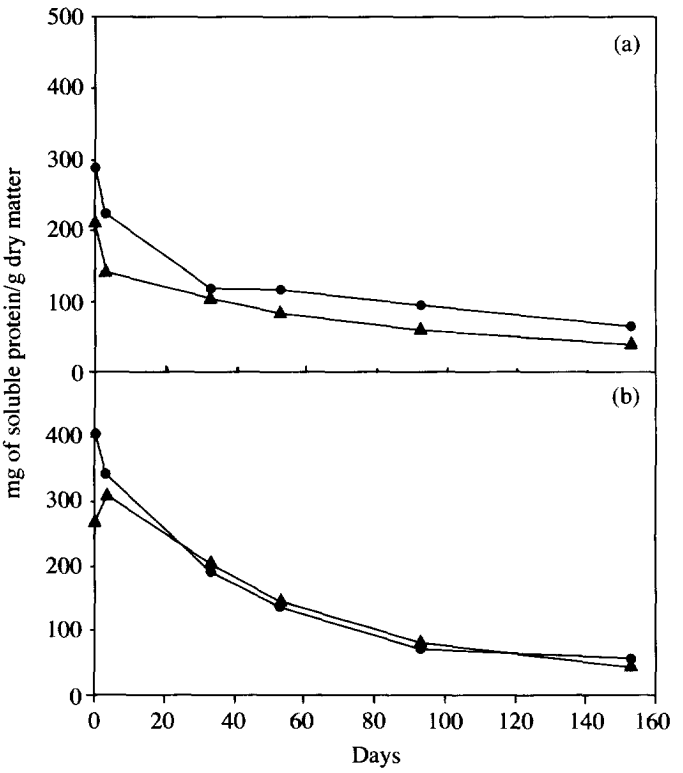

Fig. 1. Changes in the extractabilty of sarcoplasmic (a) and myofibrillar (b) proteins from *Semitendinosus* (●) and *Rectus femoris* (▲) muscles during the ripening of Spanish dried beef 'cecina'.

The changes of protein solubility in cecina may be attributed to the presence of NaCl initially added to the curing mixture. Nevertheless, the synergistic action of the salt with factors such as pH and dehydration, should not be forgotten. The formation of resistant structures may also make the extraction of proteins more difficult (Toldrá *et al.*, 1993; Córdoba *et al.*, 1994).

Identification of proteins

The following proteins in *Semitendinosus* and *Rectus femoris* muscles of the cecinas studied (Fig. 2) were identified by SDS-PAGE: myosin heavy chain (200 kda), desmin (56 kda), actin (45 kda), troponin T (37 kda), tropomyosin (35 kda), myosin LC1 (27 kda), troponin I (26 kda), troponin C (22 kda), myosin LC2 (19 kda) and myosin LC3 (16 kda). Furthermore, the two bands of molecular weight greater than 200 kda could correspond to nebulin and titin. The bands of molecular weight 126 kda, 117 kda and 93 kda could not be identified (Fig. 2).

The myofibrillar proteins undergo changes during ripening. There is a progressive reduction of the myosin heavy chain, troponin C and light myosin chain 2. The most marked decrease in these proteins seems to occur after the smoking phase. The progressive disappearance of myosin heavy chain and other myofibrillar proteins has also been

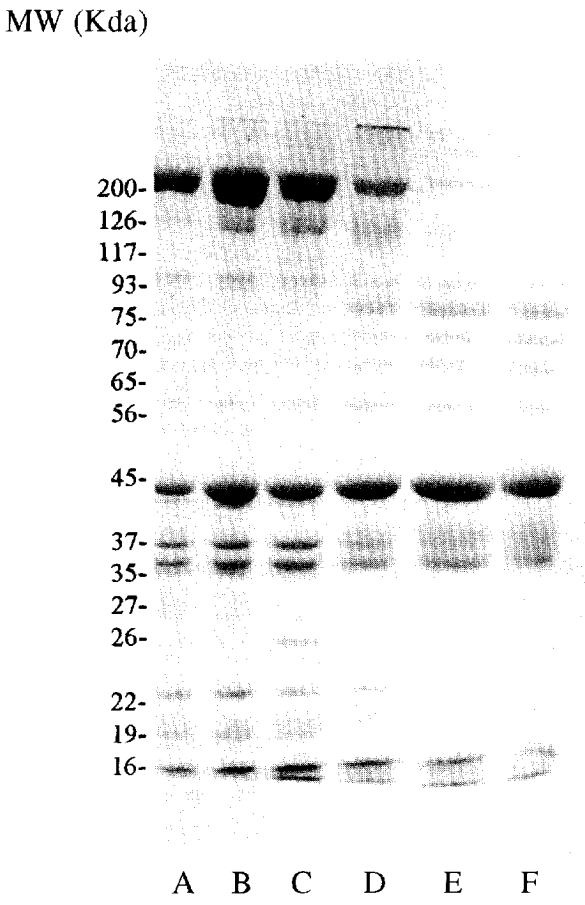

Fig. 2. Electrophoretograms of myofibrillar protein extracts obtained from *Rectus femoris* muscle at each phase of processing (A, presalting; B, salting; C, post-salting; D, smoking; E, drying; F, ripening).

observed in dry-cured hams (De Prado, 1988; Toldrá *et al.*, 1992, 1993; Córdoba *et al.*, 1994) and fermented sausages (Verplaetse *et al.*, 1989; García de Fernando and Fox, 1991).

The electrophoretograms also showed a progressive increase in three compounds of MW about 75, 70 and 65 kda (Fig. 2). The appearance of compounds of MW between 50 and 100 kda has also been observed by other authors studying the influence of various parameters (pH, temperature and storage time) on the breakdown of myofibrillar proteins in fresh meat (Yates and Greaser, 1983; Koohmaraie, 1994) and during ripening of cured hams (De Prado, 1988; Córdoba *et al.*, 1994; Toldrá *et al.*, 1992, 1993). A band with mobility close to that of myosin heavy chain and estimated molecular weight of 126 kda, increased until the smoking phase, but disappeared during the final two stages of processing. The formation of a compound of similar molecular weight (122 kda) has been observed during ripening of fermented sausages (García de Fernando and Fox, 1991). The appearance of these protein fragments could result from the breakdown of myosin heavy chain and other high molecular weight proteins.

The results obtained suggest that this cured beef product undergoes intense proteolysis, which could be owing to the action of muscle proteinases and/or micro-organisms. It has been reported that in dry-cured hams cathepsins B, D, H, and L remain active after 12–15 months of processing (Sagarra *et al.* 1993; Toldrá *et al.*, 1993). The proteolysis observed in these products is attributed to muscle proteinases (Molina and Toldrá, 1992; Toldrá *et al.*, 1993; Córdoba *et al.*, 1994). The level of *Micrococcaceae* (the predominant microbiological group in cecina, 10^2 to 10^5 ufc g^{-1}) (García *et al.*, 1995) is probably too low to produce any significant proteolytic action. This has been shown in biltong, a meat product similar to cecina (Prior, 1984).

In summary, a decrease in the extractability of nearly 80% of sarcoplasmic and myofibrillar proteins was observed in the muscles studied during the ripening process of cecina, probably owing to protein denaturation and/or proteolysis. During processing several changes in the myofibrillar proteins were noted: the virtual disappearance of myosin heavy chain, troponin C and myosin light chain 2 and the appearance of three compounds of molecular weight of 75 kda, 70 kda and 65 kda, respectively.

ACKNOWLEDGEMENT

This research was supported by a grant from the Junta de Castilla y León, España, Spain.

REFERENCES

Ambanelli, G., Molinari, C., Trasatti, V. and Pezzani, G. (1968) Ricerche sulla stagionatura del prosciutto di Parma: 1. Modificazioni nelle sostanze azotate. *Industrie Conserve* **43**, 207–210.

Cantoni, C. and Cattaneo, P. (1974) Variazioni di azoto non proteico delle proteine sarcoplasmitache e miofibrillari in prosciutti freschi e stagionati. *Archivio Veterinario Italiano* **25**, 50–56.

Córdoba, J. J., Antequera, T., Ventanas, J., López-Bote, C., García, C. and Asensio, M. C. (1994) Hydrolysis and loss of extractability of proteins during ripening of Iberian ham. *Meat Science.* **37**, 217–227.

Davis, B. J. (1964) Disc electrophoresis — II. Method and application to human serum proteins. *Annals of New York Academy Science* **121**, 404–427.

De Prado, C. (1988) Maduración del jamón de cerdo Ibérico (jabugo): Fenómenos proteolíticos. Ph D. thesis, Universidad de León.

Flores, J., Bermell, S., Nieto, P. and Costell, E. (1984) Cambios químicos en las proteínas del jamón durante los procesos del curado lento y rápido y su relación con la calidad. *Revista de Agroquímica y Tecnología de Alimentos* **24**, 503–509.

García de Fernando, G. D. and Fox, P. F. (1991) Study of proteolysis during the processing of a dry fermented pork sausage. *Meat Science* **30**, 367–383.

García, I., Zumalacárregui, J. M. and Díez, V. (1995) Microbial succession and identification of *Micrococcaceae* in dried beef cecina, an intermediate moisture meat product. *Food Microbiology* **12**, 309–315.

Gornall, A. G., Bardawill, C. J. and David, M. M. (1949) Determination of serum protein by means of the biuret reaction *Journal of Biological Chemistry* **177**, 751.

Gutiérrez, E., Domínguez, M. C. and Zumalacárregui, J. M. (1988) Caracterización de la cecina de vacuno elaborada en la provincia de León. *Anales de la Facultad de Veterinaria de León* **34**, 111–118.

Helander, E. (1957) On quantitative muscle protein determination. *Acta Physiologica Scandinavica* **41**(141), 9–95.

Koohmaraie, M. (1994) Muscle proteinases and meat aging *Meat Science* **36**, 93–104.

Kotzekidou, P. and Lazarides, H. N. (1991) Microbial stability and survival of pathogens in an intermediate moisture meat product. *Lebensmittel-Wissenschaft und-Technologie* **24**, 419–423.

Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage. *Nature* **227**, 680–685.

Molina, I. and Toldrá, F. (1992) Detection of proteolytic activity in microorganisms isolated from dry-cured ham. *Journal of Food Science* **57**, 1308–1310.

Penny, Y. F., Taylor, M. A. J., Harris, A. G. and Etherington, D. J. (1985) Purification and immunological characterization of two calcium-activated neutral proteinases from rabbit skeletal muscle. *Biochimica et Biophysica Acta* **829**, 244–252.

Presidencia del Gobierno (1979) Orden de 31 de Julio de 1979. Métodos oficiales de análisis de aceites y grasas, productos cárnicos, cereales y derivados, fertilizantes, productos fitosanitarios, productos lacteos, piensos, aguas y productos derivados de la uva *B. O. E.* (29-8-1979) **207**.

Prior, B. A. (1984) Role of microorganisms in biltong flavour development. *Journal of Applied Bacteriology* **56**, 41–45.

Sagarra, C., Gil, M. and García-Regueiro, J. A. (1993) Comparison of calpain and cathepsin (B, L and D) activities during dry-cured ham processing from heavy and light large white pigs. *Journal of the Science of Food and Agriculture* **62**, 71–75.

Serrano Moreno, A. (1979) Evolución de varias microfloras y su interdependencia con las condiciones físico-químicas durante la maduración del salchichón. *Alimentaria* **100**, 39–56.

Sinell, H. J. and Hentschel, S. (1977) Zur chemischem zusammensetzung un Mikrobiologie einiger getrockneter fleischproduckte. *Fleischwirtschaft* **57**, 1317–1320.

Souci, S. W., Fachmann, W. and Kraut, H. (1974) Die Zusammensetzungder lebensmittel; Nährwert-Tabellen. Wissenschaftliche verlagsgesellschaft mbH, Stuttgart. Cited in Sinell, H. J. and Hentschel, S. (1977) *Fleischwirstchaft* **57**, 1317–1320.

Toldrá, F., Miralles, M. C. and Flores, J. (1992) Protein extractability in dry-cured ham. *Food Chemistry* **44**, 391–394.

Toldrá, F., Rico, E. and Flores, J. (1993) Cathepsin B, D, H, and L activities in the processing of dry-cured ham *Journal of the Science of Food and Agriculture* **62**, 157–161.

Verplaetse, A., Debosschere, M. and Demeyer, D. (1989) Proteolysis during dry sausage ripening. *Proc. 35th Int. Cong. Meat Science Technology*, Vol. 3, pp. 815–818. Copenhagen, Denmark.

Weber, K. and Osborn, M. (1969) The reliability of molecular weight determinations by dodecyl sulphate-poliacrylamide gel electrophoresis. *Journal of Biological Chemistry* **244**, 4406–4412.

Yates, L. D. and Greaser, M. L. (1983) Quantitative determination of myosin and actin in rabbit skeletal muscle. *Journal of Molecular Biology* **168**, 1230.