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Protein extractability in dry-cured ham

Fidel Toldrá, M.-Carmen Miralles & José Flores

Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Jaime Roig, 11 - 46010 Valencia, Spain

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Different procedures for the extraction of myofibrillar and total proteins of both raw and dry-cured ham have been assayed. Three solvents—(1) 0·1 M phosphate buffer + 1·1 M KI, pH = 7·4, (2) 0·1 M tris/HCl + 1 % (w/v) sodium dodecyl sulphate, pH = 7·0 and (3) 8 M urea + 1 % (v/v) β-mercaptoethanol–were used for myofibrillar proteins extraction and the homogenates compared quantitatively and by electrophoresis. Solvent (3) gave a higher protein recovery from dry-cured ham than solvent (2) although the electrophoretic patterns showed similar profiles. One relevant fact was the appearance of a 150 KDa fragment and numerous fragments in the 50–100 KDa region. No myosin band was observed.

INTRODUCTION

The effect of pH and salt concentration upon protein extractability in raw meat has been studied by many authors (Helander, 1957; Klement *et al.*, 1973; Samejima & Wolfe, 1976; Yates *et al.*, 1983; Richardson & Jones, 1987; Parsons & Knight, 1990). However, few works have attempted to explain the problems associated with protein extraction in dry-cured meat products. Many biochemical changes are known to take place during the dry-cure processes. One of the major changes reported is an intense proteolysis, expressed as non-protein nitrogen or total soluble nitrogen (Bellatti *et al.* 1983; Flores *et al.*, 1984; Astiasaran *et al.*, 1990).

Proteins also experience a partial denaturation during the dry-curing process resulting in a decrease in solubility (Klement *et al.*, 1973; Wardlaw *et al.*, 1973; Samejima & Wolfe, 1976; Ledward, 1981; Babiker, 1985). Some of these proteins, not correctly solubilized, may have been proteolized. Thus, the selection of the most appropriate solvents for muscle myofibrillar proteins extraction is essential (Yates *et al.*, 1983; Wu & Smith, 1987; An *et al.*, 1988).

The objective of this work is to extract as much sarcoplasmic and myofibrillar protein as possible (both denaturated and undenaturated) from dry-cured ham without hydrolyzing these proteins in order for them to be electrophoretically evaluated and compared with the fresh meat proteins.

MATERIALS AND METHODS

Muscle samples

Samples of M. Biceps femoris were removed from six-month-old pigs and processed about 8–10 h post mortem. The same muscle was also removed from dry-cured hams which were obtained from different commercial processors. These hams had undergone the traditional Spanish process consisting of salting (10 days at 3°C) followed by a post-salting storage period (22 days at 5°C), a resting period (2 months at 6–9°C) and drying (5 months at 13–14°C). The characteristics of these hams are shown in Table 1. Water activity (*Aw*) was determined at 25°C using a Humidat RC (Novasina, Zürich, Switzerland). Moisture, pH and salt content were determined according to official methods (Presidencia del Gobierno, 1979).

Protein extraction

The samples (4 g diluted 1 : 10 w/v with 0·03M potassium phosphate, pH = 7.4) were extracted at 2°C

Table 1. Characteristics of the hams used in the study[a]

	Raw hams	Dry-cured hams
Moisture (%)	74 ± 1	63 ± 1
Water activity, *Aw*	99·5 ± 0·5	89 ± 1
pH	5·6 ± 0·1	5·8 ± 0·1
NaCl (%)	—	4·9 ± 0·5

[a] Means of 12 hams ± standard error

according to the classical method of Helander (1957). The same samples were also extracted according to a modified method which consisted of 2 min extraction with the same buffer in a Stomacher (London, UK) homogenizer followed by centrifugation at 10 000*g* for 20 min instead of a 3 h extraction and centrifugation at 1500*g* for 20 min. The soluble protein in this low ionic strength buffer was considered to be sarcoplasmic protein.

In both cases, the pellet resulting from the sarcoplasmic protein extraction and washing was extracted again at 2°C for 2 min in a Stomach homogenizer with three types of solutions (dilution 1 : 10, w/v): (1) 0·1M phosphate buffer + 1.1M KI, pH = 7·4; (2) 0·1M tris/HCl + 1 % (w/v) sodium dodecyl sulphate (SDS), pH = 7·0; and (3) 8M urea + 1 % (v/v) β-mercaptoethanol. The homogenates were centrifuged under refrigeration at 10 000g for 20 min and the supernatants, containing the myofibrillar proteins, collected.

Total protein extracts were also prepared by homogenizing 4 g of sample in 40 ml (dilution 1 : 10 w/v) of each type of solution for 2 min in a Stomacher homogenizer and centrifuging the homogenate at 10 000g for 20 min.

Protein determination

The protein concentration was determined by the method of Bradford (1976) using bovine serum albumin (BSA) as the standard. The interference of SDS and urea in samples was corrected.

Electrophoresis

Protein samples were prepared for electrophoresis as described by Toldrá *et al.* (1990). Each was mixed in a ratio of 2 : 5 : 1 (v/v) with 0·5M tris/HCl, pH = 8·0, containing 5% (w/v) SDS, 14·5% (v/v) β-mercaptoethanol, 30% (v/v) glycerol and 0·03% (v/v) bromophenol blue. 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 fixed by soaking in a solution of 25% (v/v) isopropanol and 12·5% (w/v) trichloroacetic acid for 30 min, washed and stained overnight in 0·01% (w/v) Coomassie Brilliant Blue R 250 (Weber & Osborn, 1969). The gels were destained with distilled water until the background was clear and scanned at 632·8 nm in a LKB 2202 Ultroscan Laser Densitometer with absorbance range from 0 to 1.

Standard proteins (high and low molecular weight) from Pharmacia (Uppsala, Sweden) were simultaneously run for protein identification. These standard proteins were: thyroglobulin (330 000), ferritin (22 000), serum albumin (67 000), catalase (60 000), lactate dehydrogenase (36 000) and phosphorylase b (94 000), ovalbumin (43 000), carbonic anhydrase (30 000), trypsin inhibitor (20 100) and α-lactalbumin (14 400).

RESULTS AND DISCUSSION

The modification introduced to the classical Helander method, which drastically reduces the total time necessary for sample processing, increased significantly ($P < 0.05$) the concentration of solubilized sarcoplasmic and myofibrillar proteins in raw uncured ham (see Table 2). Kuchroo and Fox (1982) also observed a good efficiency of the Stomacher homogenizer when extracting cheese proteins, probably due to a very vigorous blending action. The changes of protein solubility in dry-cured hams may be attributed to the presence of NaCl initially added in the curing mixture and, on the other hand, the denaturing effect of heat created by the curing process as observed by many authors (Klement *et al.*, 1973; Wardlaw *et al.*, 1973; Astiasaran *et al.*, 1990) with fermented sausages. Thus, the extraction of myofibrillar proteins from dry-cured ham is very low when using the Helander-method (see Table 2) and many proteins do not appear on electrophoresis (Figs. 1 (E, F) and 2 (b)) as compared to raw ham (Figs. 1 (A, B) and 2 (a)).

Table 2. The effect of different solvents upon protein extractability of fresh meat and dry-cured ham

Extraction system	Protein concentration (mg/g dry sample)[a]					
	Raw ham			Dry-cured ham		
	Sarcoplasmic	Myofibrillar	Total proteins	Sarcoplasmic	Myofibrillar	Total proteins
Helander	61 ± 7[a]	67 ± 7[c]	131 ± 10[e]	68 ± 8[b]	23 ± 3[h]	89 ± 5[j]
Modified Helander	68 ± 7[b]	85 ± 20[d]	149 ± 15[f]	65 ± 14[b]	21 ± 3[h]	105 ± 12[k]
Tris/SDS[b]	68 ± 7[b]	62 ± 9[c]	136 ± 12[e,g]	65 ± 14[b]	74 ± 23[c,d]	149 ± 19[f,g]
Urea/β-mercaptoethanol[b]	68 ± 7[b]	90 ± 17[d]	142 ± 12[f,g]	65 ± 14[b]	160 ± 21[i]	211 ± 21[l]

[a] Mean of 12 samples ± standard error. Any two means having the same letters are not significantly different at $P < 0.05$.
[b] Helander modified method used for the extraction of sarcoplasmic proteins.

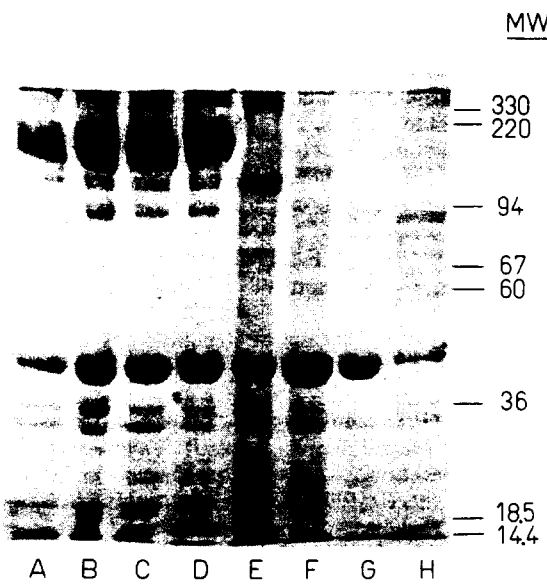

Fig. 1. SDS-polyacrylamide gel electrophoretic patterns of myofibrillar proteins from both raw (lanes A, B, C and D) and dry-cured hams (lanes E, F, G and H). Lanes A and E correspond to high ionic strength extracts by the classical Helander method while lanes B and F are by modified Helander. Lanes C and G correspond to tris/SDS homogenates and lanes D and H to urea/β-mercaptoethanol homogenates.

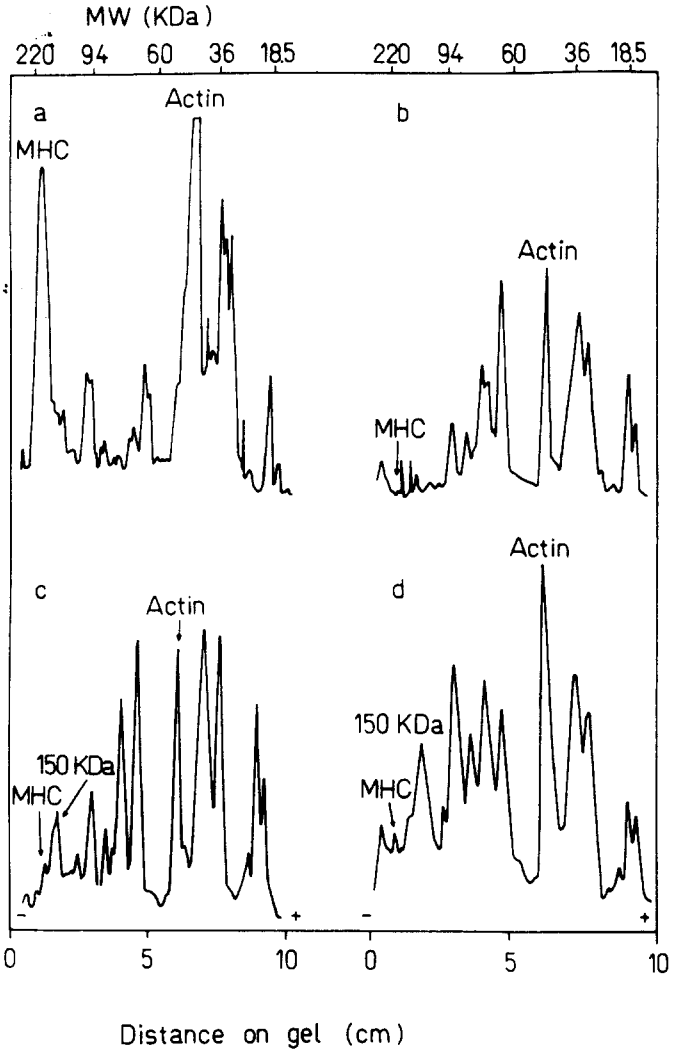

Fig. 2. Densitometry patterns of total protein extracts: (a) high ionic strength extract by modified Helander method from raw ham; and (b) dry-cured ham; (c) and (d) tris/SDS and urea/β-mercaptoethanol homogenates, respectively, from dry-cured hams.

Solvents for protein extraction, other than phosphate buffer, were also assessed. SDS is a common solvent which produces a net negative charge on protein molecules, destabilizing structures by rupture of hydrogen bonds and hydrophobic regions (Ledward, 1981; An *et al.*, 1988). This solvent did not give a significant ($P < 0{\cdot}05$) improvement in myofibrillar protein recovery from raw ham than high ionic strength phosphate buffer (see Table 2) but showed a significant increase ($P < 0{\cdot}05$) in protein solubility of dry-cured hams which was also reflected in the high number of protein fragments (Figs 1 (G) and 2 (c)) in the range 50–100 KDa and a peak of an unidentified protein corresponding to 150 KDa.

Urea is another denaturing agent which reduces hydrophobic interactions and hydrogen bonds (An *et al.*, 1988; Taylor & Etherington, 1991) while β-mercaptoethanol splits disulphide linkages (Ledward, 1981). This solvent was found to be the most effective for protein extraction from dry-cured hams, giving the highest protein recovery (see Table 2). The electrophoretic patterns (see Figs. 1 (H) and 2 (d)) showed similar profiles to the SDS extracts but, in this case, higher quantities of α-actinin and actin were recovered. Numerous protein fragments in the range 50–100 KDa were also found as well as the unidentified protein of 150 KDa.

Extracts prepared using all solvents gave rise to similar electrophoretic patterns with fresh pork myofibrillar proteins (Figs 1 (A, D)). As previously indicated, the main differences were found when comparing the dry-cured ham extracts. No myosin heavy chain band was detected and a protein fragment of about 150 KDa became apparent in the dry-cured ham extracts (see Figs 1 (E, F) and 2 (c, d)) which could be the result of myosin heavy chain degradation (Schwartz & Bird, 1977; Penny & Ferguson-Pryce, 1979; Robbins *et al.*, 1979). As suggested by Yates *et al.* (1983), the rest of myosin molecules may be found in the 50–100 KDa region where numerous protein fragments have appeared (see Figs 1 (E–H) and 2(b–d). Thus, a breakdown of high MW proteins, such as myosin, would take place during the dry-curing process. One possibility is the breakdown taking place as a result of proteolytic action. In fact, cathepsins B, D, H and L have been found to be active even after eight months of dry-curing (Toldrá & Etherington, 1988). Another possibility is a loss of solubility by

partial denaturation due to the dry-curing itself (Wardlaw *et al.*, 1973; Klement *et al.*, 1973; Astiassaran *et al.*, 1990). It is also possible that oxidation reactions, perhaps catalyzed by heavy metals present as contaminants in the cure mixture, could form crosslinks between molecules making their extraction more difficult (Knight & Parsons, 1988).

In summary, the use of denaturing solvents such as SDS or, preferably, urea/β-mercaptoethanol is necessary to extract myofibrillar proteins from dry-cured ham. The disappearance of myosin and the appearance of an unidentified 150 KDa protein and numerous protein fragments in the 50–100 KDa region constitute a very interesting fact. The authors' research, at this moment is focused on the extraction of myofibrillar proteins from hams at different stages of the dry-curing process in order to elucidate at which stage enzymic proteolysis is most intense and when disappearance of myosin starts.

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