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# Evaluation of microbial proteolysis in meat products by capillary electrophoresis

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**Aims:** The available methods for evaluating proteolysis in meat products, particularly the contribution of micro-organisms, are expensive, time-consuming and require an unacceptable sample size. To minimize these problems, two capillary electrophoresis-based methods have been developed.

**Methods and Results:** Six Gram-positive, catalase-positive cocci, four moulds and three yeasts, isolated from dry-cured ham, were tested on sterile pork slices. Using the Capillary Gel Electrophoresis (CGE) method, changes in sarcoplasmic and myofibrillar proteins due to endogenous and microbial enzymes were detected. The Capillary Zone Electrophoresis (CZE) analysis allowed evaluation of bulk changes by micro-organisms in soluble nitrogen compounds.

**Conclusions:** CGE analysis of myofibrillar proteins and CZE determination of soluble nitrogen compounds have proved to be valuable tools for evaluating proteolytic activity of endogenous and microbial origin.

**Significance and Impact of the Study:** The CGE and CZE methods developed can be used for a rapid and sensitive analysis of proteolysis in meat products.

## INTRODUCTION

Most dry-cured meat products are obtained after a ripening time of 1–24 months. During this time, sarcoplasmic and myofibrillar proteins undergo deep proteolytic changes that influence the texture and flavour of the products. Both endogenous and microbial enzymes may play a decisive role in these changes.

Proteolysis by endogenous enzymes during the processing of dry-cured ham has been studied by Toldrá *et al*. (1993). Cathepsins B, H and L are partially active during the whole processing of ham (Toldrá and Etherington 1988). However, there is little information about the microbial contribution to proteolysis in dry-cured ham. This is partly due to the lack of an appropriate technique for evaluating the changes caused by micro-organisms. There are methods based on SDS-PAGE and HPLC analyses to evaluate the proteolytic ability of micro-organisms (Molina and Toldrá 1992; LeBlanc *et al*. 1994; Rodríguez *et al*. 1998). However, these methods are expensive, time-consuming (usually the analysis takes one to two days), and the required sample size is unacceptable for routine control of some meat products such as dry-cured ham. Several rapid and sensitive methods for analysing proteins by capillary electrophoresis have been reported in recent years (Zeece 1992; Cancalon 1995; Lindeberg 1996). As analysis by capillary electrophoresis is relatively quick, usually taking about 1 h, and only small amounts of reagents and sample are needed, it could be used to evaluate the proteolytic activity on meat products.

The aim of this work was to design a method for evaluating the proteolytic changes in dry-cured meat products by capillary electrophoresis that could be used to study the contribution of micro-organisms and the progress of ripening.

## MATERIALS AND METHODS

### Microbial cultures

Six Gram-positive, catalase-positive cocci, four moulds and three yeasts (Table 1), isolated from dry-cured ham (Rodríguez *et al*. 1994, 1996; Núñez *et al*. 1996a,b) and selected for their proteolytic activity against myosin (Rodríguez *et al*. 1998), were used. To obtain the stock

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**Table 1** Sarcoplasmic proteins in fresh pork slices and in sterile and inoculated samples after 30 d of incubation at 25 °C

| | Proteins (MW in kDa) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 15 | 23 | 24 | 30 | 38 | 41 | 46 | 49 | 55 | 92 | 182 |
| Fresh | 0·21 ± 0·07$^{a}$ | 0·75 ± 0·01$^{a}$ | 1·21 ± 0·13$^{a}$ | 0·98 ± 0·47$^{a}$ | 2·36 ± 0·21$^{a}$ | 1·32 ± 0·05$^{a}$ | 0·45 ± 0·24$^{a}$ | 1·01 ± 0·10$^{a}$ | 2·30 ± 0·19$^{a}$ | 0·37 ± 0·27$^{a}$ | 1·46 ± 0·85$^{ab}$ |
| Sterile | n.d.*$^{b}$ | 0·46 ± 0·07$^{ab}$ | 1·14 ± 0·12$^{a}$ | 0·17 ± 0·11$^{b}$ | 2·24 ± 0·67$^{a}$ | n.d.$^{b}$ | 0·27 ± 0·08$^{ab}$ | n.d.$^{b}$ | 1·31 ± 0·44$^{a}$ | n.d.$^{b}$ | 1·51 ± 0·72$^{ab}$ |
| *Staphylococcus xylosus* 9AA8 | n.d.$^{b}$ | 0·47 ± 0·02$^{ab}$ | 0·61 ± 0·09$^{bc}$ | 0·04 ± 0·02$^{b}$ | 1·61 ± 0·02$^{ab}$ | n.d.$^{b}$ | 0·17 ± 0·05$^{ab}$ | n.d.$^{b}$ | 0·56 ± 0·07$^{b}$ | n.d.$^{b}$ | 1·19 ± 0·47$^{ab}$ |
| *Staphylococcus xylosus* 5EA | n.d.$^{b}$ | 0·58 ± 0·01$^{ab}$ | 0·91 ± 0·03$^{ab}$ | n.d.$^{b}$ | 2·39 ± 0·05$^{a}$ | n.d.$^{b}$ | 0·28 ± 0·06$^{ab}$ | n.d.$^{b}$ | 0·87 ± 0·22$^{ab}$ | n.d.$^{b}$ | 2·16 ± 0·28$^{ab}$ |
| *Staphylococcus cohnii* 8CD | n.d.$^{b}$ | 0·45 ± 0·15$^{ab}$ | 0·68 ± 0·25$^{abc}$ | 0·05 ± 0·01$^{b}$ | 1·65 ± 0·59$^{ab}$ | n.d.$^{b}$ | 0·18 ± 0·10$^{ab}$ | n.d.$^{b}$ | 0·70 ± 0·10$^{ab}$ | n.d.$^{b}$ | 1·40 ± 0·41$^{a}$ |
| *Staphylococcus equorum* 8AB2 | n.d.$^{b}$ | 0·63 ± 0·16$^{a}$ | 0·81 ± 0·13$^{ab}$ | 0·02 ± 0·01$^{b}$ | 1·83 ± 0·23$^{ab}$ | n.d.$^{b}$ | 0·31 ± 0·01$^{ab}$ | n.d.$^{b}$ | 0·81 ± 0·07$^{ab}$ | n.d.$^{b}$ | 2·13 ± 0·31$^{a}$ |
| *Staphylococcus equorum* 1AB2 | n.d.$^{b}$ | 0·57 ± 0·30$^{ab}$ | 0·66 ± 0·23$^{abc}$ | 0·06 ± 0·06$^{b}$ | 2·23 ± 1·04$^{a}$ | n.d.$^{b}$ | 0·38 ± 0·38$^{a}$ | n.d.$^{b}$ | 0·81 ± 0·43$^{ab}$ | n.d.$^{b}$ | 2·17 ± 0·98$^{ab}$ |
| *Tetragenococcus halophila* M1A2 | n.d.$^{b}$ | 0·34 ± 0·11$^{ab}$ | 0·63 ± 0·14$^{bc}$ | n.d.$^{b}$ | 0·94 ± 0·31$^{b}$ | n.d.$^{b}$ | 0·16 ± 0·09$^{ab}$ | n.d.$^{b}$ | 0·60 ± 0·31$^{b}$ | n.d.$^{b}$ | 0·70 ± 0·21$^{b}$ |
| *Penicillium chrysogenum* 222 | n.d.$^{b}$ | 0·51 ± 0·06$^{ab}$ | 0·66 ± 0·06$^{bc}$ | 0·11 ± 0·06$^{b}$ | 1·38 ± 0·24$^{ab}$ | n.d.$^{b}$ | 0·14 ± 0·04$^{ab}$ | n.d.$^{b}$ | 0·53 ± 0·01$^{b}$ | n.d.$^{b}$ | 0·31 ± 0·06$^{ab}$ |
| *Penicillium commune* 131 | n.d.$^{b}$ | 0·54 ± 0·09$^{ab}$ | 0·62 ± 0·22$^{bc}$ | 0·10 ± 0·06$^{b}$ | 1·65 ± 0·71$^{ab}$ | n.d.$^{b}$ | 0·16 ± 0·06$^{ab}$ | n.d.$^{b}$ | 0·81 ± 0·46$^{ab}$ | n.d.$^{b}$ | 1·42 ± 0·45$^{ab}$ |
| *Paecylomyces variotti* 351 | n.d.$^{b}$ | 0·51 ± 0·04$^{ab}$ | 0·59 ± 0·05$^{bc}$ | 0·08 ± 0·09$^{b}$ | 1·77 ± 0·43$^{ab}$ | n.d.$^{b}$ | 0·17 ± 0·05$^{ab}$ | n.d.$^{b}$ | 0·77 ± 0·19$^{ab}$ | n.d.$^{b}$ | 1·68 ± 0·42$^{ab}$ |
| *Eurotium repens* 131 | n.d.$^{b}$ | 0·48 ± 0·27$^{ab}$ | 0·49 ± 0·25$^{bc}$ | 0·04 ± 0·04$^{b}$ | 1·02 ± 0·49$^{ab}$ | n.d.$^{b}$ | 0·03 ± 0·02$^{b}$ | n.d.$^{b}$ | 0·89 ± 0·49$^{ab}$ | n.d.$^{b}$ | 0·69 ± 0·02$^{ab}$ |
| *Debaryomyces hansenii* 344 | n.d.$^{b}$ | 0·38 ± 0·15$^{ab}$ | 0·48 ± 0·22$^{bc}$ | 0·06 ± 0·01$^{b}$ | 1·30 ± 0·55$^{ab}$ | n.d.$^{b}$ | 0·17 ± 0·06$^{ab}$ | n.d.$^{b}$ | 0·54 ± 0·15$^{b}$ | n.d.$^{b}$ | 1·28 ± 0·55$^{ab}$ |
| *Debaryomyces hansenii* 345 | n.d.$^{b}$ | 0·32 ± 0·04$^{b}$ | 0·38 ± 0·13$^{c}$ | 0·04 ± 0·04$^{b}$ | 1·39 ± 0·32$^{ab}$ | n.d.$^{b}$ | 0·12 ± 0·03$^{ab}$ | n.d.$^{b}$ | 0·48 ± 0·10$^{b}$ | n.d.$^{b}$ | 1·61 ± 0·96$^{a}$ |
| *Debaryomyces hansenii* 131 | n.d.$^{b}$ | 0·40 ± 0·01$^{ab}$ | 0·53 ± 0·06$^{bc}$ | 0·10 ± 0·04$^{b}$ | 1·62 ± 0·52$^{ab}$ | n.d.$^{b}$ | 0·14 ± 0·06$^{ab}$ | n.d.$^{b}$ | 0·51 ± 0·04$^{b}$ | n.d.$^{b}$ | 1.96 ± 1.04$^{a}$ |

Values are given as arbitrary area units; *not detected.

For a given protein (column), values showing different letters as superscript are significantly different ($P < 0·05$).

 

cultures, cocci were cultured in Nutrient Broth (Oxoid) for 24 h at 31°C, and moulds and yeast in Malt Extract Agar (Oxoid) for 7 days at 25°C. Serial dilutions in 0·1% Peptone Broth (Oxoid) from pure cultures were made and those containing about $10^6$ cfu $ml^{-1}$ were used for meat inoculation.

### Preparation of meat slices

Pork sirloins were removed from carcasses and the exterior of the muscle was sterilized by searing as described by Dainty and Hibbard (1980). The burnt tissues were removed using sterile instruments in a laminar flow cabinet Bio Flow II (Telstar, Tarrasa, Spain). The sterile muscle was then cut into 1 cm thick slices, and samples of about 50 g were placed in pre-sterilized 250 ml conical flasks (Rodríguez *et al*. 1998). Sterile NaCl was added at 5% w/w to the meat, to obtain conditions similar to those on hams during the ripening process.

Sterile, salted meat slices were inoculated with 0·1 ml of the dilution containing $10^6$ cfu $ml^{-1}$ of the selected strain.

### Sampling

Inoculated samples were incubated at 25°C for 30 days. Both fresh and incubated sterile slices were tested as controls. All samples were taken in triplicate. Total viable counts were determined on Plate Count Agar (Oxoid) and samples were taken for analysis by capillary electrophoresis.

### Analysis of sarcoplasmic and myofibrillar proteins by Capillary Gel Electrophoresis (CGE)

Sarcoplasmic proteins were extracted from 2 g sample with 40 ml 0·03 mol $l^{-1}$, pH 7·1, sodium phosphate buffer (Córdoba *et al*. 1994). The extract was centrifuged at 8000 *g* for 15 min and the supernatant fluid was filtered through a 0·45 $\mu$m filter. Myofibrillar proteins were extracted with 40 ml 0·12 mol $l^{-1}$ Tris-HCl, pH 10·6, 1% SDS, from the resultant pellet. The extract was then centrifuged and filtered as indicated above.

For protein denaturalization, 200 $\mu$l of the sarcoplasmic extract were mixed with 100 $\mu$l 0·12 mol $l^{-1}$ Tris-HCl, pH 6·6, 1% SDS and 15 $\mu$l 2-mercaptoethanol (Sigma), while for myofibrillar proteins, 200 $\mu$l of extract were mixed with 15 $\mu$l of 2-mercaptoethanol. Orange G solution was added to both sarcoplasmic and myofibrillar extracts as internal standard. Denaturalized extracts were incubated at 100°C for 10 min. Incubated extracts were put on ice until analysis.

Analysis of sarcoplasmic and myofibrillar proteins was performed on an automated PACE 2050 controlled by a PACE System Gold (Beckman Instruments, Inc., Palo Alto, CA, USA). A coated capillary column of 75 $\mu$m diameter and 37 cm total length (Beckman) was used. The capillary column was conditioned with 0·1 M HCl for 1 min, purified water for 1 min and buffer gel SDS 14–200 kit from Beckman for 5 min, before every run. Samples were injected by a 30 s pressure injection. Capillary column temperature was kept at 25°C. Electrophoretic separations were performed at 8 kV for 45 min. Proteins were monitored at 214 nm. To calculate the molecular weight of the different bands obtained, the coefficient of relative time mobility to orange G was compared with those of two different protein standards: (i) myoglobin, glyceraldehyde phosphate dehydrogenase, creatine kinase and phosphorylase B (Sigma); (ii) $\alpha$-lactalbumin, carbonic anhydrase, ovalbumin, bovine serum albumin, phosphorylase B, $\beta$-galactosidase and myosin (Beckman).

### Analysis of soluble nitrogen fractions by Capillary Zone Electrophoresis (CZE)

For extraction and analysis of the soluble nitrogen fraction, 2 g samples were extracted with 40 ml 0·03 mol $l^{-1}$ sodium phosphate buffer (Córdoba *et al*. 1994) at pH 6·6, 7·4 and 8·3. The mixture obtained was centrifuged at 8000 g for 15 min and the supernatant fluid filtered through a 0·45 $\mu$m filter.

For CZE analysis, the same equipment was used, with a 75 $\mu$m diameter, 27 cm total length, uncoated, fused, silica capillary from Supelco (Tecknocroma, Barcelona, Spain). The capillary was conditioned by flushing for 3 min with 1 mol $l^{-1}$ NaOH, 1 min with purified water and 5 min with 0·03 mol $l^{-1}$ sodium phosphate buffer at the pH used for extraction. The last buffer was also used as electrolyte buffer. Samples were injected by 1 s pressure injection. Electrophoretic separations were performed at 3 kV for 30 min and 25°C. Nitrogen compounds were monitored at 214 nm. Myoglobin, creatine kinase, aldolase, and a mixture of the free amino acids ASP, GLU, ASN, GLY, SER, GLN, ARG, HIS, THR, ALA, PRO, TYR, VAL, MET, LEU, ILE, PHE, TRP and LYS (Sigma), were used as standards.

### Statistical analysis

Statistical analysis of data was carried out by one-way analysis of variance, and means were separated by Tukey's honest significant difference test using a StatGraphics software package from Statistical Graphics Corp. (Rockville, MD, USA).

## RESULTS

### Sarcoplasmic proteins by CGE

A total of 11 peaks, ranging from 15 to 182 kDa, was obtained in sarcoplasmic extract from sterile fresh pork (Fig. 1a). Peaks of 15, 41, 49 and 92 kDa were not detected

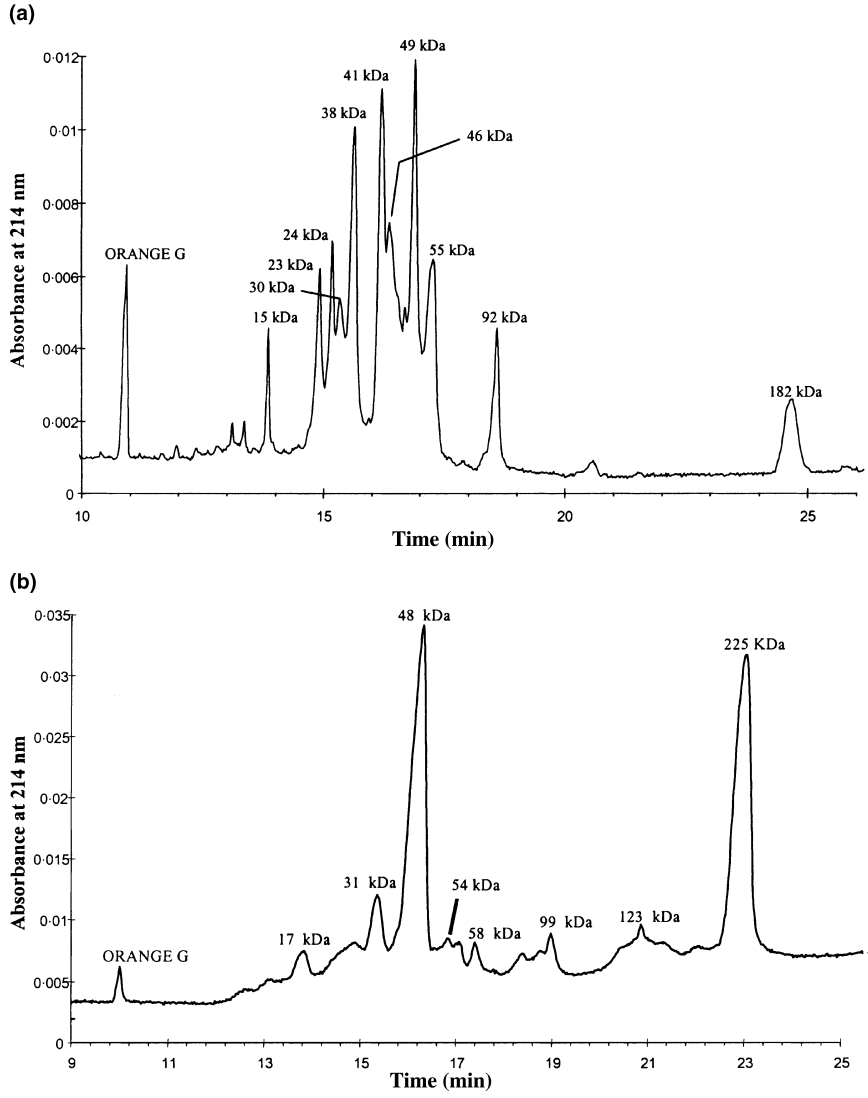


**Fig. 1** Electropherograms of sarcoplasmic (a) and myofibrillar (b) proteins of fresh pork

in any of the incubated samples. In addition, the peak of 30 kDa showed a significant ($P < 0{\cdot}05$) reduction in every incubated sample after 30 days of incubation time.

Sterile samples always showed the highest mean values of the incubated samples for every sarcoplasmic protein. However, these proteins did not show large differences amongst inoculated samples (Table 1). The peak of 24 kDa seemed to be sensitive to the inoculated strains, except for most *Staphylococcus* strains. The remaining proteins were attacked to a limited extent by just a few strains. Peaks of 23, 38 and 46 kDa were hydrolysed by just one strain, and the peak of 55 kDa, by six of the 13 strains tested (Table 1).

## Myofibrillar proteins by CGE

Eight peaks ranging from 17 to 225 kDa were detected in fresh pork by CGE (Fig. 1b). The two main peaks of this fraction were identified as H-meromyosin (225 kDa) and actin (48 kDa), according to the analysis of standards. An additional peak of 42 kDa was found in most incubated samples (Table 2).

Actin showed a sharp reduction in most batches ($P < 0{\cdot}05$) during incubation (Table 2). On the other hand, the 54 kDa peak increased in two batches.

Most micro-organisms tested decreased ($P < 0{\cdot}05$) the area of the 17 kDa peak compared with sterile incubated samples (Table 2), but none reduced the peaks of 31, 42 and 58 kDa. Moreover, when compared with sterile incubated samples, only two micro-organisms, *Debaryomyces hansenii* 344 and *Penicillium chrysogenum* 222, induced a significant decrease in the H-meromyosin peak, and four (*P. chrysogenum* 222, *Staphylococcus equorum* 8AB2, *Tetragenococcus halophila* M1A2, and *Paecilomyces variotti* 351) reduced the peak of actin. From all these, only *P. chrysogenum* 222 showed major

**Table 2** Myofibrillar proteins in fresh pork slices and in sterile and inoculated samples after 30 d of incubation at 25°C

| | Proteins (MW in kDa) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 17 | 31 | 42 | 48 (actin) | 54 | 58 | 99 | 123 | 225 (H-meromyosin) |
| Fresh | 0·50 ± 0·10$^{ab}$ | 0·69 ± 0·49$^{a}$ | n.d.$^{b}$* | 8·01 ± 1·15$^{ab}$ | 0·09 ± 0·07$^{b}$ | 0·21 ± 0·07 | 0·64 ± 0·07$^{ab}$ | 0·57 ± 0·31$^{abc}$ | 9·43 ± 0·89$^{a}$ |
| Sterile | 1·01 ± 0·03$^{a}$ | 0·44 ± 0·02$^{ab}$ | 3·49 ± 0·69$^{ab}$ | 6·73 ± 0·17$^{bc}$ | 2·11 ± 0·22$^{a}$ | 0·20 ± 0·02 | 0·62 ± 0·10$^{ab}$ | 1·15 ± 0·39$^{a}$ | 3·03 ± 0·44$^{a}$ |
| *Staphylococcus xylosus* 9AA8 | 0·19 ± 0·01$^{b}$ | 0·47 ± 0·14$^{ab}$ | 2·05 ± 2·05$^{ab}$ | 4·75 ± 0·31$^{cd}$ | 0·71 ± 0·46$^{ab}$ | 0·08 ± 0·06 | 0·55 ± 0·03$^{abcd}$ | 0·94 ± 0·14$^{ab}$ | 1·46 ± 0·50$^{ab}$ |
| *Staphylococcus xylosus* 5EA | 0·13 ± 0·13$^{b}$ | 0·19 ± 0·19$^{ab}$ | 1·57 ± 1·57$^{b}$ | 5·12 ± 0·47$^{cd}$ | 0·89 ± 0·16$^{ab}$ | 0·15 ± 0·04 | 0·22 ± 0·01$^{cde}$ | 0·32 ± 0·18$^{abc}$ | 1·52 ± 0·69$^{ab}$ |
| *Staphylococcus cohnii* 8CD | 0·30 ± 0·08$^{b}$ | 0·23 ± 0·07$^{ab}$ | n.d.$^{b}$ | 9·46 ± 1·71$^{a}$ | 0·70 ± 0·70$^{ab}$ | 0·62 ± 0·62 | 0·22 ± 0·22$^{cde}$ | n.d.$^{c}$ | 2·45 ± 0·75$^{ab}$ |
| *Staphylococcus equorum* 8AB2 | 0·31 ± 0·01$^{b}$ | 0·14 ± 0·01$^{ab}$ | 2·97 ± 2·97$^{ab}$ | 3·83 ± 1·03$^{de}$ | 0·64 ± 0·08$^{ab}$ | 0·23 ± 0·23 | 0·57 ± 0·07$^{abcd}$ | 0·15 ± 0·15$^{bc}$ | 0·48 ± 0·22$^{ab}$ |
| *Staphylococcus equorum* 1AB2 | 0·52 ± 0·11$^{ab}$ | 0·38 ± 0·17$^{ab}$ | 1·83 ± 1·83$^{b}$ | 5·33 ± 1·49$^{cd}$ | 0·28 ± 0·11$^{b}$ | 0·19 ± 0·19 | 0·61 ± 0·03$^{ab}$ | 0·57 ± 0·57$^{abc}$ | 2·07 ± 2·07$^{ab}$ |
| *Tetragenococcus halophila* M1A2 | 0·48 ± 0·27$^{ab}$ | 0·04 ± 0·04$^{b}$ | 0·70 ± 0·70$^{b}$ | 2·22 ± 0·32$^{e}$ | n.d.$^{b}$ | 0·27 ± 0·07 | 0·21 ± 0·02$^{de}$ | 0·70 ± 0·48$^{ab}$ | 1·39 ± 1·39$^{ab}$ |
| *Penicillium chrysogenum* 222 | 0·06 ± 0·06$^{b}$ | 0·18 ± 0·11$^{ab}$ | 0·33 ± 0·30$^{b}$ | 1·44 ± 0·67$^{e}$ | n.d.$^{b}$ | 0·04 ± 0·04 | n.d.$^{e}$ | 0·31 ± 0·11$^{abc}$ | n.d.$^{b}$ |
| *Penicillium commune* 131 | 0·39 ± 0·07$^{b}$ | 0·50 ± 0·00$^{ab}$ | 1·64 ± 1·64$^{b}$ | 8·35 ± 0·08$^{ab}$ | 0·40 ± 0·21$^{b}$ | 0·39 ± 0·23 | 0·41 ± 0·00$^{abcd}$ | 0·60 ± 0·35$^{abc}$ | 1·11 ± 1·11$^{ab}$ |
| Paecylomyces *variotti* 351 | 0·25 ± 0·00$^{b}$ | 0·26 ± 0·10$^{ab}$ | 0·86 ± 0·86$^{b}$ | 3·97 ± 0·58$^{de}$ | 0·59 ± 0·17$^{ab}$ | n.d. | 0·32 ± 0·03$^{abcde}$ | 0·57 ± 0·35$^{abc}$ | 1·92 ± 1·00$^{ab}$ |
| Eurotium *repens* 131 | 1·13 ± 0·22$^{a}$ | n.d.$^{b}$ | 0·42 ± 0·42$^{b}$ | 9·48 ± 0·77$^{a}$ | 0·75 ± 0·49$^{ab}$ | 0·15 ± 0·15 | 0·58 ± 0·17$^{abc}$ | 0·32 ± 0·32$^{abc}$ | 2·24 ± 1·68$^{ab}$ |
| *Debaryomyces hansenii* 344 | 0·34 ± 0·05$^{b}$ | 0·39 ± 0·39$^{ab}$ | n.d.$^{b}$ | 5·71 ± 0·32$^{cd}$ | 0·71 ± 0·54$^{ab}$ | 0·05 ± 0·05 | 0·28 ± 0·28$^{bcde}$ | 0·19 ± 0·19$^{bc}$ | n.d.$^{b}$ |
| *Debaryomyces hansenii* 345 | 0·09 ± 0·09$^{b}$ | 0·07 ± 0·07$^{b}$ | 2·19 ± 2·19$^{ab}$ | 6·12 ± 0·19$^{bc}$ | 0·07 ± 0·03$^{b}$ | 0·26 ± 0·26 | 0·25 ± 0·25$^{bcde}$ | 0·66 ± 0·12$^{abc}$ | 0·86 ± 0·11$^{ab}$ |
| *Debaryomyces hansenii* 131 | 0·56 ± 0·15$^{ab}$ | 0·34 ± 0·03$^{ab}$ | 6·72 ± 0·55$^{a}$ | 5·23 ± 0·33$^{cd}$ | 1·46 ± 0·60$^{a}$ | 0·50 ± 0·50 | 0·68 ± 0·06$^{a}$ | 0·17 ± 0·17$^{bc}$ | 0·38 ± 0·20$^{ab}$ |

Values are given as arbitrary area units; *not detected.

For a given protein (column), values showing different letters as superscript are significantly different ($P < 0·05$).

changes in most peaks, including the two main myofibrillar proteins.

### Soluble nitrogen fractions by CZE

The pH value of the sodium phosphate buffer had a marked influence on the resolution of the soluble compounds by CZE (Fig. 2). The best results were obtained at pH 8·3. Under these conditions, seven peaks (A–G) were observed in fresh pork. An additional peak (H) was also detected in samples inoculated with some micro-organisms. The analysis of standards revealed that free amino acids elute in peaks A and E, and myoglobin in peak B.

Incubation of sterile samples at 25°C for 30 days led to increases ($P < 0{\cdot}05$) in peaks C and F, but reductions in peaks A and B (Table 3). Several inoculated samples showed a further reduction in peak B, while peaks C and F decreased to the levels of fresh pork, or even lower. On the other hand, peaks A, D, E, G and H were affected by just a few strains. These strains increased some of the peaks (A, E and H) to levels even higher than those of fresh pork, while peaks D and G decreased to the lowest values obtained.

Samples inoculated with *P. chrysogenum* 222 showed significant increases in peaks containing amino acids (A and E), and decreases in peaks B, C, F and G. Other strains, such as *Penicillium commune* 131, *Eurotium repens* 131 and *Staphylococcus equorum* 8AB2, caused significant decreases in at least four peaks. However, only strains *Staph. equorum* 1AB2 and *P. commune* 131 caused increases in peak A, but did not increase peak E.

## DISCUSSION

CGE analysis for sarcoplasmic proteins of fresh pork revealed 11 peaks. Those of 15, 38, 41 and 92 kDa were identified, respectively, as myoglobin, glyceraldehyde phosphate dehydrogenase, creatine kinase and phosphorylase B, according to standards. The remaining peaks of 23, 24, 30, 46, 49 and 55 kDa could be, according to their molecular weight, myokinase, triose phosphate isomerase, phosphoglycerate mutase, enolase, phosphoglucose isomerase and pyruvate kinase (Scopes and Penni 1971; McCornick *et al.* 1988). Data regarding compounds of a molecular weight around 182 kDa have not been reported in pork sarcoplasmic proteins. As this peak showed a molecular weight higher than those of sarcoplasmic proteins, it could derive from hydrolysis of myofibrillar proteins.

The reduction in some of the sarcoplasmic proteins during incubation of sterile samples must be attributed to the proteolytic activity of endogenous enzymes, such as alanyl, leucyl and tyrosyl aminopeptidases, which remain active in meat (Lauffart and Mantle 1988; Nishimura *et al.* 1988a,b). Very little additional effect was observed in sarcoplasmic proteins from inoculated samples. However, the reduction in the peaks of 24 and 55 kDa denotes hydrolysis of sarcoplasmic proteins, particularly by fungal strains.

CGE of the myofibrillar proteins showed a total of eight peaks. Those of 225 and 48 kDa were the major myofibrillar proteins H-meromyosin and actin. Peaks of 17, 31, 99 and 123 kDa show molecular weights similar to those of L-meromyosin, tropomyosin, α-actinin and C protein, respectively (Maruyama 1985). A peak of a molecular weight close to 55 kDa has been reported from hydrolysis of myofibrillar proteins during meat maturation (Robson *et al.* 1981). This peak was detected in a higher amount in sterile samples after incubation than in fresh pork. Thus, it could have originated from hydrolysis of myosin, which showed a deep reduction during incubation. Therefore, myosin and the peak of 55 kDa could be of great interest for evaluating the proteolysis extension in pork.

In most inoculated samples, some myofibrillar proteins suffered an additional reduction compared with those observed in uninoculated samples. The effect of the strains tested on these proteins is higher than that observed in sarcoplasmic proteins. As myofibrillar proteins seem to be more sensitive to proteolysis by micro-organisms isolated from dry-cured ham, the analysis of this protein fraction could be a reliable method for quantifying the proteolytic ability of micro-organisms. *Penicillium chrysogenum* 222 showed the highest proteolytic activity, particularly on the two main myofibrillar proteins, as has been shown previously by SDS-PAGE (Rodríguez *et al.* 1998).

With the CGE method developed in this work, it is possible to detect changes in sarcoplasmic and myofibrillar proteins, irrespective of their endogenous or microbial origin. This method allowed the detection of even more changes than those observed by SDS-PAGE (Rodríguez *et al.* 1998). Thus, the information obtained by SDS-PAGE in 1–2 days can be obtained by CGE analysis in less than 1 h, and more simply.

In addition, a capillary electrophoresis method based on CZE of soluble nitrogenous compounds was optimized to quantify proteolysis of microbial origin with 0·03 mol $l^{-1}$ sodium phosphate buffer at pH 8·3. This method allowed the detection of bulk changes in sarcoplasmic proteins, free amino acids and peptides derived from proteolysis in meat samples.

Incubation of sterile pork samples caused increases in peaks C and F as well as a reduction in peak B, denoting proteolysis from endogenous enzymes.

In inoculated samples, changes detected in soluble compounds by CZE were sharper than those observed in sarcoplasmic proteins by CGE. The higher sensitivity of CZE to evaluate changes in protein extracts is due to the ability of this method to detect small peptides and free amino acids derived from protein hydrolysis. The increase

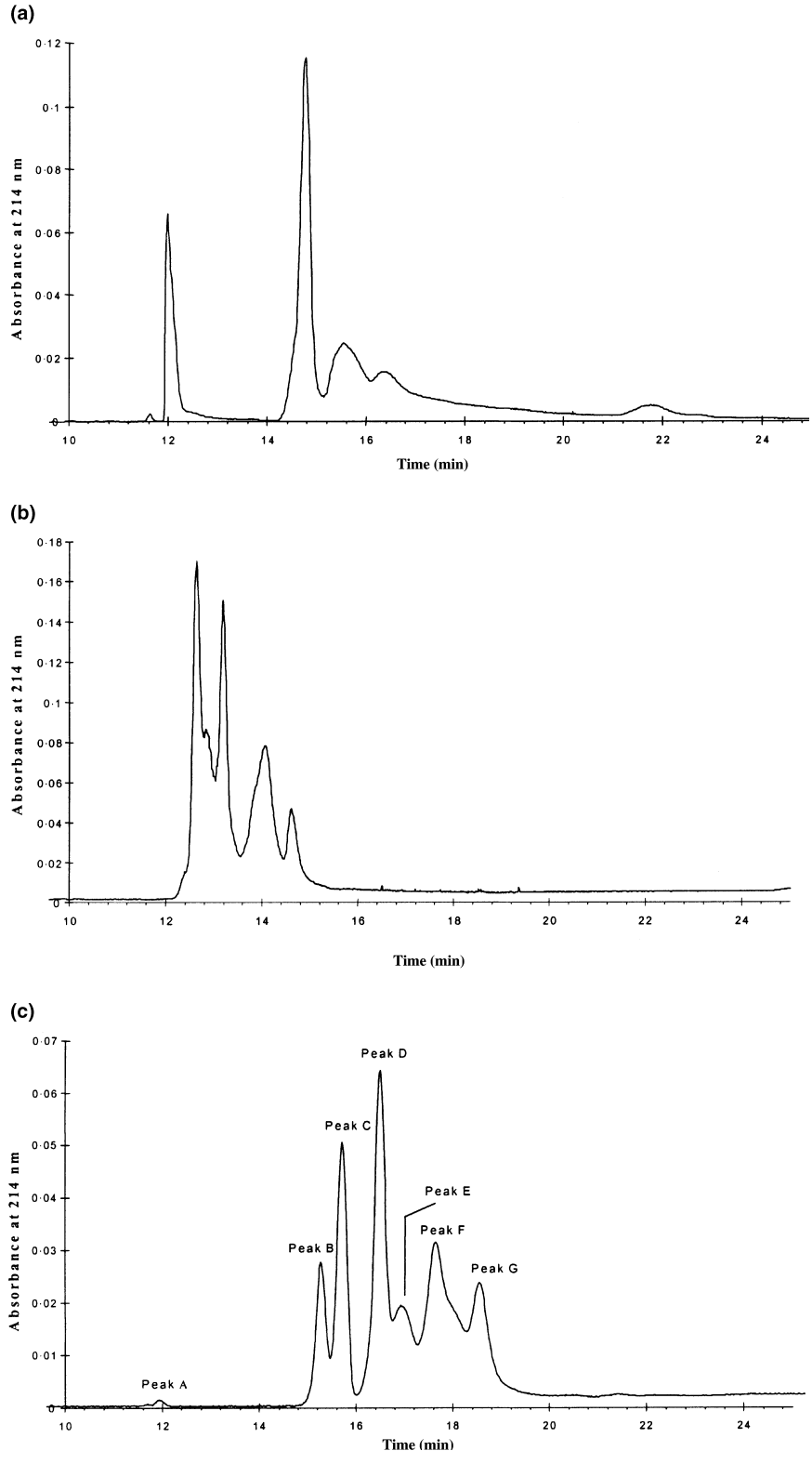


**Fig. 2** Electropherograms of soluble nitrogen fractions in phosphate buffer 0·03 mol $l^{-1}$, pH 6·6 (a), 7·4 (b), and 8·3 (c)

in peaks A and E observed in samples inoculated with *P. chrysogenum* 222 revealed the increase in free amino acids, as has been reported by HPLC analysis of pork inoculated with this mould strain (Rodríguez *et al.* 1998). These changes caused by *P. chrysogenum* have to be derived mainly from hydrolysis of myosin and actin.

**Table 3** Soluble nitrogenous compounds in phosphate buffer 0·03M, pH 8·3 in fresh pork slices and in sterile and inoculated samples after 30 d of incubation at 25 °C

| | Peaks | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H |
| Fresh | 0·34 ± 0·05$^{bc}$ | 5·76 ± 0·77$^{a}$ | 5·95 ± 0·68$^{cd}$ | 8·49 ± 0·54$^{a}$ | 0·36 ± 0·36$^{b}$ | 2·41 ± 0·76$^{b}$ | 3·47 ± 0·16$^{a}$ | n.d.$^{c}$ |
| Sterile | 0·06 ± 0·09$^{d}$ | 2·95 ± 1·06$^{bc}$ | 7·80 ± 1·33$^{ab}$ | 7·99 ± 3·30$^{ab}$ | 1·00 ± 0·57$^{b}$ | 6·83 ± 2·81$^{a}$ | 3·69 ± 1·36$^{a}$ | n.d.$^{c}$ |
| *Staphylococcus xylosus* 9AA8 | n.d.$^{d}$* | 5·20 ± 0·39$^{a}$ | 8·38 ± 1·39$^{a}$ | 6·87 ± 3·54$^{bc}$ | 1·44 ± 0·65$^{b}$ | 0·16 ± 0·16$^{c}$ | 4·69 ± 1·35$^{a}$ | 0·69 ± 0·14$^{a}$ |
| *Staphylococcus xylosus* 5EA | n.d.$^{d}$ | 0·72 ± 0·05$^{e}$ | 3·48 ± 0·01$^{de}$ | 2·44 ± 0·15$^{c}$ | 0·74 ± 0·02$^{b}$ | 0·09 ± 0·08$^{c}$ | 2·24 ± 0·73$^{ab}$ | 0·48 ± 0·01$^{abc}$ |
| *Staphylococcus cohnii* 8 CD | n.d.$^{d}$ | 1·86 ± 0·04$^{cd}$ | 7·01 ± 0·33$^{bc}$ | 5·31 ± 1·06$^{bc}$ | 0·51 ± 0·24$^{b}$ | 0·38 ± 0·22$^{c}$ | 3·42 ± 0·15$^{a}$ | 0·10 ± 0·10$^{c}$ |
| *Staphylococcus equorum* 8AB2 | n.d.$^{d}$ | 0·42 ± 0·14$^{e}$ | 5·17 ± 0·41$^{de}$ | 3·68 ± 0·14$^{c}$ | 0·91 ± 0·07$^{b}$ | n.d.$^{c}$ | 2·99 ± 0·45$^{ab}$ | 0·09 ± 0·11$^{c}$ |
| *Staphylococcus equorum* 1AB2 | 0·27 ± 0·19$^{bc}$ | 4·31 ± 0·83$^{ab}$ | 7·51 ± 0·76$^{ab}$ | 8·90 ± 1·15$^{a}$ | 1·61 ± 0·14$^{b}$ | 1·42 ± 1·26$^{c}$ | 4·70 ± 1·17$^{a}$ | 0·17 ± 0·24$^{c}$ |
| *Tetragenococcus halophila* M1A2 | n.d.$^{d}$ | 5·25 ± 0·18$^{a}$ | 6·42 ± 0·24$^{cd}$ | 9·48 ± 0·38$^{a}$ | 1·16 ± 0·29$^{b}$ | 3·96 ± 0·71$^{b}$ | 2·71 ± 0·32$^{ab}$ | n.d.$^{c}$ |
| *Penicillium chrysogenum* 222 | 1·77 ± 0·15$^{a}$ | 1·41 ± 0·30$^{de}$ | 5·83 ± 0·41$^{de}$ | 5·00 ± 1·53$^{bc}$ | 7·65 ± 2·07$^{a}$ | 0·42 ± 0·30$^{c}$ | 1·17 ± 0·54$^{b}$ | n.d.$^{c}$ |
| *Penicillium commune* 131 | 0·40 ± 0·12$^{b}$ | 1·64 ± 0·15$^{cd}$ | 5·24 ± 0·81$^{de}$ | 5·96 ± 1·23$^{bc}$ | 1·72 ± 0·23$^{b}$ | 0·23 ± 0·16$^{c}$ | 2·29 ± 0·33$^{ab}$ | 0·62 ± 0·27$^{ab}$ |
| *Paecylomyces variotti* 351 | 0·18 ± 0·04$^{cd}$ | 1·56 ± 0·16$^{de}$ | 4·53 ± 0·36$^{de}$ | 5·14 ± 0·70$^{bc}$ | 1·18 ± 0·06$^{b}$ | 0·76 ± 0·63$^{c}$ | 3·88 ± 1·07$^{a}$ | n.d.$^{c}$ |
| *Eurotium repens* 131 | n.d.$^{d}$ | 1·11 ± 0·93$^{de}$ | 3·21 ± 2·61$^{e}$ | 5·99 ± 4·14$^{bc}$ | 1·53 ± 0·05$^{b}$ | 1·28 ± 1·28$^{c}$ | 3·13 ± 1·87$^{ab}$ | 0·62 ± 0·13$^{ab}$ |
| *Debaryomyces hansenii* 344 | n.d.$^{d}$ | 1·28 ± 0·49$^{de}$ | 6·44 ± 2·30$^{cd}$ | 6·29 ± 2·68$^{bc}$ | 0·96 ± 0·62$^{b}$ | 0·33 ± 0·42$^{c}$ | 3·10 ± 0·89$^{ab}$ | 0·22 ± 0·12$^{bc}$ |
| *Debaryomyces hansenii* 345 | n.d.$^{d}$ | 1·33 ± 0·61$^{cd}$ | 6·01 ± 1·15$^{cd}$ | 4·61 ± 0·74$^{bc}$ | 0·89 ± 0·33$^{b}$ | 0·36 ± 0·31$^{c}$ | 4·27 ± 1·10$^{a}$ | 0·28 ± 0·39$^{abc}$ |
| *Debaryomyces hansenii* 131 | 0·13 ± 0·17$^{cd}$ | 2·00 ± 1·29$^{bc}$ | 5·47 ± 0·63$^{de}$ | 6·22 ± 0·46$^{bc}$ | 0·97 ± 0·22$^{b}$ | 0·24 ± 0·25$^{c}$ | 2·56 ± 0·19$^{ab}$ | 0·38 ± 0·31$^{abc}$ |

Values are given as arbitrary units; *not detected.
For a given peak (column), values showing different letters as superscript are significantly different ($P < 0·05$).

CZE analysis of nitrogen compounds could be of great interest for quantifying the degree of proteolysis in meat products, as well as for evaluating the proteolytic activity of microbial origin. In addition, CZE analysis is faster than CGE and it is also cheaper, since only a single fused silica capillary is needed.

In conclusion, CGE analysis of myofibrillar proteins, and CZE analysis of soluble nitrogen compounds, have proved to be valuable tools for evaluating proteolysis in meat products, and for determining proteolytic activity of microbial origin.

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