ELSEVIER

Food Chemistry 76 (2002) 377–384

Food Chemistry

www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods Section

# Capillary zone electrophoresis as a method for identification of golden kinglip (*Genypterus blacodes*) species during frozen storage

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Received 13 February 2001; received in revised form 11 June 2001; accepted 11 June 2001

**Abstract**

Capillary zone electrophoresis (CZE) was used to study the effect of frozen storage on the electrophoretic patterns of sarcoplasmic proteins (SAR) extracted from fillets of golden kinglip (*Genypterus blacodes*) species using fresh species as a reference. CZE was developed using phosphoric acid at pH 1.5 and an uncoated silica capillary (50 μm i.d.×75 cm). This condition provided an optimum separation of fish SAR in less than 22 min. Protein profiles were reproducible among individuals belonging to the same species. There was less variation in relative migration time than in peak area percentage for each frozen temperature and storage time assayed. Profiles were independent of fish freshness degree: frozen fish showed the same protein profile as their fresh counterpart. CZE allows correct identification of frozen golden kinglip fish species stored at −18 or −30 °C from 0 to 180 days. 

*Keywords:* Capillary zone electrophoresis; Fish species identification; Sarcoplasmic protein; Frozen fish

## 1. Introduction

Analysis of sarcoplasmic proteins (SAR) present in the water extracts of fish muscle is of major interest for species identification (LeBlanc, Singh, & LeBlanc, 1994) because of labelling regulations, quality, and pricing policies imposed by various countries (Mermelstein, 1993).

A variety of analytical methods, based on the separation of SAR, have been employed to identify species. Although gel electrophoresis of proteins is widely used as a separation technique, it is labour-intensive, time-consuming and semiquantitative (McCormick, 1988). High-performance liquid chromatography (HPLC) has provided species profiles for raw fish (Armstrong, Leach, & Wyllie, 1992). In recent years, capillary zone electrophoresis (CZE) has permitted rapid, high-resolution separations of proteins (McCormick, 1988) and represents a significant departure from conventional gel electrophoretic separations. CZE is very useful for the separation of proteins and peptides since complete resolution can often be obtained for analytes differing by only one amino acid substituent (Beckmann, 1994).

The analysis of SAR by CZE has been used to identify and differentiate fresh flatfish species (Gallardo, Sotelo, Piñeiro, & Pérez-Martin, 1995) and to control changes in SAR after 4 months of frozen storage at different temperatures (LeBlanc et al., 1994).

Freezing and frozen storage of fish cause textural changes which often decrease water retention of muscle proteins, particularly in meagre fish (Ragnarsson & Regenstein, 1989). This is probably due to the enzymatic breakdown of trimethylamine oxide (TMAO) to dimethylamine (DMA) and formaldehyde (FA). FA is then believed to react with fish proteins to accelerate the undesirable texture changes (Hebard, Flick, & Martin, 1982). Such changes could affect the characteristic electrophoretic patterns, making species identification difficult and leading to wrong results (Larraín, Pérez, & Vinagre, 1999).

The aim of this work is to demonstrate that CZE permits correct identification of frozen golden kinglip (*Genypterus blacodes*) at −18 °C and −30 °C for up to 180 days, by monitoring changes in SAR pattern.

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0308-8146/02/$ - see front matter 
PII: S0308-8146(01)00263-1

## 2. Materials and methods

### 2.1. Preparation of samples and frozen storage

Three authentic specimens of golden kinglip named K, L and M were obtained from the Chilean coast in fresh-freezing state. Fish fillets were prepared from each specimen, cut in similar pieces, packed in Poliethylene bags, labelled and stored at −18 and −30 °C. Every 30 days of frozen storage at each temperature, one sample was removed and analysed, up to 180 days of storage.

### 2.2. SAR extraction and quantification

Fresh or frozen fillets (5 g) were homogenized with 15 ml of distilled and deionized (Milli-Q) water at 4 °C to solubilize the SAR, using an OMNI homogenizer (1 min at 25,000 rpm). The mixture was then centrifuged at 10,000 ***g*** for 20 min in a Sorvall centrifuge at 4 °C. Supernatant was taken and protein content determined by Warburg method (Warburg & Christian, 1941). Extracts were frozen at −30 °C. Each specimen was analysed by duplicate. Before CZE analysis, the extracts were filtered through a 0.22 μm filter and diluted with water to achieve the protein concentration of 2.5–3.0 mg/ml used in all analyses.

### 2.3. CZE of SAR

Analysis by CZE was performed using Capillary Ion Analyzer (Waters, Milford, MA, USA) with Millenium software (Waters) for data handling. UV detection was accomplished at 185 nm (Hg lamp) and the sample was introduced by hydrodynamic injection (10 cm height). A fused-silica capillary (68 cm to the detector) of 50 μm i.d. was used. The capillary was conditioned with 0.5 M NaOH for 2 min, Milli-Q water for 1 min, 0.1 M NaOH for 10 min, Milli-Q water for 3 min and finally with 150 mM phosphoric acid, pH 1.5, which was also employed as electrolyte with 5.48 mM *n*-octylamine, 5% methanol and 10% acetonitrile (Valenzuela et al., 1999). The electrophoretic analysis was performed at 20 kV and 23 °C with sample injections for 10 s.

Between runs, the capillary was rinsed with 0.2 M NaOH for 4 min and with water for 3 min, followed by a recondition step with the running electrolyte for 7 min.

### 2.4. Statistical analysis

The Statgraphic plus 4.0 program was used to perform one-way ANOVA ($p \leqslant 0.05$) on the results. Comparisons of means after ANOVA were performed using the least squared difference (LSD) method.

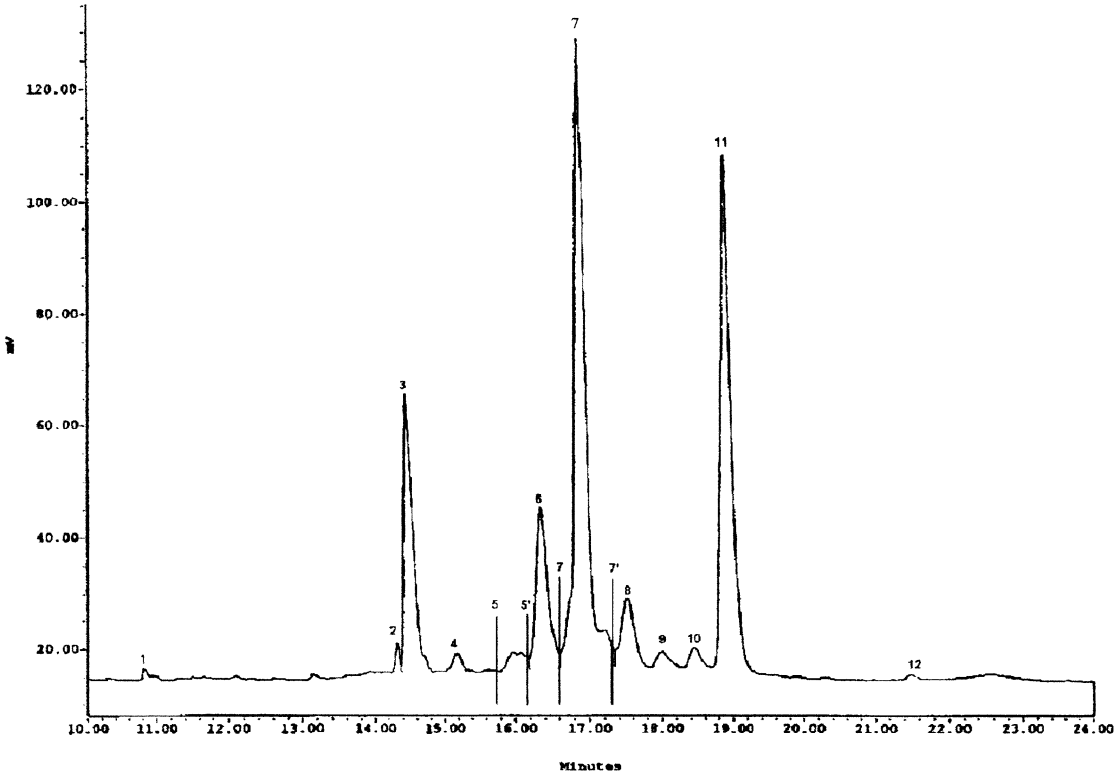


Fig. 1. Electropherograms of SAR from fresh fish maintained at 0 °C. Peaks are labelled as in Table 1.

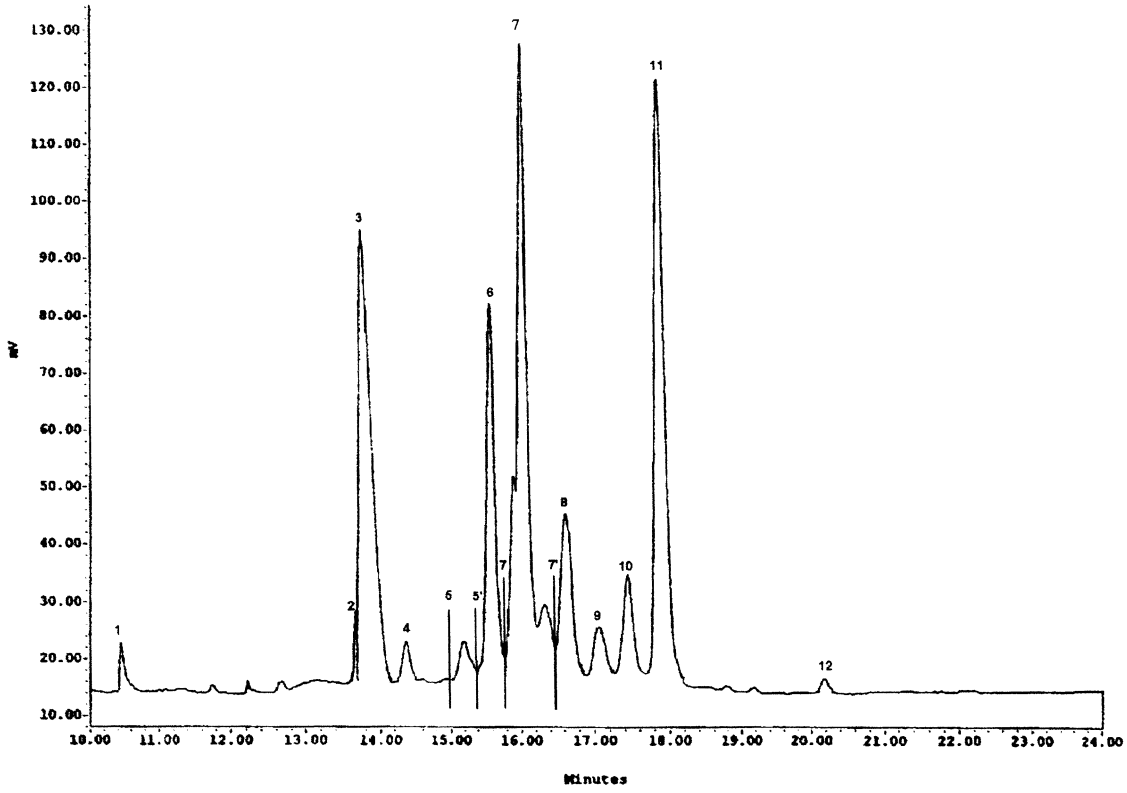


Fig. 2. Electropherograms of SAR from fish maintained at −18 °C for 180 days. Peaks are labelled as in Table 1.

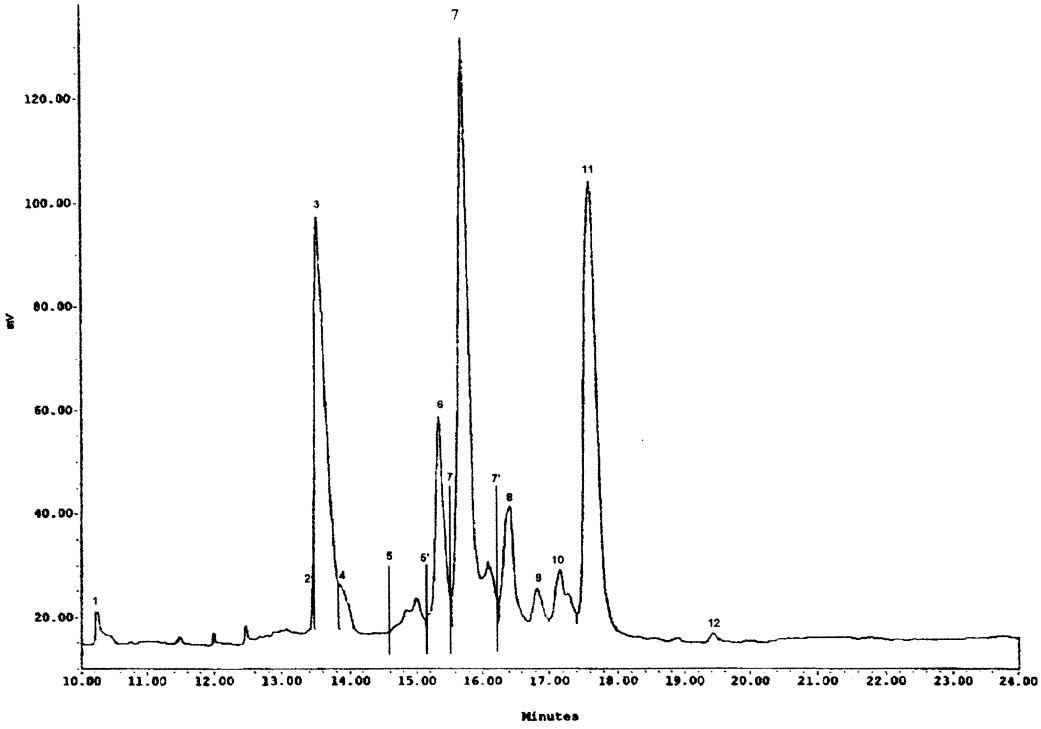


Fig. 3. Electropherograms of SAR from fish maintained at −30 °C for 180 days. Peaks are labelled as in Table 2.

Table 1
RMT and AP for each peak of SAR at −18 °C with corresponding standard deviations between individuals ($n = 3$, RMT relative migration time, AP percentage area of the peak)

| Time | Peak | 1 | 2 | 3 | 4 | 5 | 5′ | 6 | 7 | 7′ | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T0 | RMT | 0.00 | 3.49 | 3.60 | 4.36 | 4.94 | 5.56 | 5.52 | 5.76 | 6.50 | 6.67 | 7.16 | 7.61 | 8.02 | 10.63 |
| T0 | SD | 0.00 | 0.03 | 0.02 | 0.02 | 0.07 | 0.28 | 0.08 | 0.09 | 0.09 | 0.10 | 0.12 | 0.13 | 0.09 | 0.09 |
| T0 | AP | 1.16 | 3.93 | 100 | 5.37 | 11.2 | – | 52.6 | 187.8 | – | 37.8 | 5.28 | 16.3 | 181 | 1.53 |
| T0 | SD | 0.55 | 0.91 | 0.00 | 1.02 | 1.20 | – | 5.00 | 2.25 | – | 1.75 | 1.59 | 7.36 | 7.68 | 0.23 |
| T30 | RMT | 0.00 | 3.42 | 3.49 | 4.48 | 4.84 | 5.27 | 5.43 | 5.61 | 6.39 | 6.56 | 7.05 | 7.48 | 7.92 | 11.00 |
| T30 | SD | 0.00 | 0.18 | 0.21 | 0.38 | 0.26 | 0.31 | 0.34 | 0.34 | 0.42 | 0.42 | 0.48 | 0.42 | 0.46 | 0.49 |
| T30 | AP | 1.72 | 2.40 | 100 | 4.19 | 6.90 | – | 60.7 | 157 | – | 24.0 | 6.82 | 12.9 | 195 | 1.10 |
| T30 | SD | 1.07 | 2.09 | 0.00 | 0.96 | 3.12 | – | 1.05 | 7.31 | – | 4.96 | 1.64 | 6.63 | 5.55 | 0.28 |
| T60 | RMT | 0.00 | 3.23 | 3.33 | 4.03 | 4.52 | 4.98 | 5.18 | 5.41 | 6.12 | 6.24 | 6.73 | 7.19 | 7.61 | 10.6 |
| T60 | SD | 0.00 | 0.14 | 0.16 | 0.33 | 0.38 | 0.28 | 0.22 | 0.23 | 0.19 | 0.23 | 0.29 | 0.30 | 0.30 | 0.26 |
| T60 | AP | 1.70 | 4.10 | 100 | 4.66 | 6.43 | – | 58.1 | 172 | – | 28.2 | 4.91 | 14.3 | 201 | 1.71 |
| T60 | SD | 0.97 | 2.62 | 0.00 | 1.14 | 1.58 | – | 5.25 | 8.08 | – | 7.60 | 1.89 | 4.11 | 0.93 | 0.21 |
| T90 | RMT | 0.00 | 3.17 | 3.26 | 4.20 | 4.51 | 4.94 | 5.11 | 5.32 | 5.98 | 6.12 | 6.62 | 7.07 | 7.47 | 10.2 |
| T90 | SD | 0.00 | 0.29 | 0.30 | 0.23 | 0.33 | 0.44 | 0.41 | 0.42 | 0.50 | 0.52 | 0.50 | 0.50 | 0.49 | 0.52 |
| T90 | AP | 2.20 | 1.56 | 100 | 5.09 | 9.33 | – | 60.4 | 145 | – | 27.6 | 4.62 | 14.8 | 149 | 1.68 |
| T90 | SD | 1.26 | 0.81 | 0.00 | 0.73 | 5.60 | – | 7.42 | 8.84 | – | 5.00 | 1.28 | 3.04 | 5.43 | 0.27 |
| T120 | RMT | 0.00 | 3.05 | 3.12 | 3.97 | 4.29 | 4.65 | 4.81 | 4.97 | 5.63 | 5.78 | 6.21 | 6.63 | 6.98 | 9.17 |
| T120 | SD | 0.00 | 0.13 | 0.11 | 0.38 | 0.15 | 0.16 | 0.18 | 0.17 | 0.23 | 0.26 | 0.26 | 0.31 | 0.22 | 0.22 |
| T120 | AP | 2.08 | 1.66 | 100 | 3.54 | 4.57 | – | 57.1 | 124 | – | 26.8 | 4.41 | 9.93 | 147 | 1.91 |
| T120 | SD | 1.36 | 0.98 | 0.00 | 1.14 | 2.01 | – | 7.60 | 4.98 | – | 5.99 | 0.72 | 1.28 | 4.68 | 0.62 |
| T150 | RMT | 0.00 | 3.06 | 3.16 | 4.24 | 4.42 | 4.72 | 4.89 | 5.08 | 5.72 | 5.82 | 6.27 | 6.72 | 7.11 | 9.82 |
| T150 | SD | 0.00 | 0.05 | 0.05 | 0.04 | 0.05 | 0.07 | 0.08 | 0.07 | 0.11 | 0.13 | 0.13 | 0.10 | 0.09 | 0.01 |
| T150 | AP | 2.96 | 1.90 | 100 | 7.26 | 6.05 | – | 58.1 | 149 | – | 24.1 | 5.88 | 24.7 | 98.3 | 2.02 |
| T150 | SD | 1.95 | 1.17 | 0.00 | 1.23 | 1.14 | – | 1.28 | 6.85 | – | 5.73 | 0.56 | 1.64 | 8.52 | 0.42 |
| T180 | RMT | 0.00 | 3.19 | 3.26 | 3.89 | 4.54 | 4.92 | 5.08 | 5.28 | 5.97 | 6.11 | 6.56 | 6.93 | 7.33 | 9.67 |
| T180 | SD | 0.00 | 0.02 | 0.02 | 0.09 | 0.04 | 0.02 | 0.02 | 0.03 | 0.04 | 0.05 | 0.03 | 0.05 | 0.04 | 0.20 |
| T180 | AP | 3.21 | 2.10 | 100 | 5.27 | 7.35 | – | 56.0 | 141 | – | 24.6 | 7.09 | 20.1 | 96.2 | 2.23 |
| T180 | SD | 1.72 | 0.95 | 0.00 | 0.69 | 2.04 | – | 8.03 | 0.56 | – | 6.31 | 2.19 | 5.09 | 7.68 | 0.39 |

## 3. Results and discussion

### 3.1. CZE separation of SAR

Most fish SAR have pI values higher than 2.44 (McCormick, 1988). These proteins could be positively charged at pH 1.5 and move to the cathode, where the detector window is located. Another advantage of using a low pH is reducing the adsorption of proteins to the capillary wall (Karger, Cohen, & Guttman, 1989). This condition of pH using an uncoated capillary, and in the presence of additives contained in the electrolyte, provided optimum separation of fish SAR in less than 22 min total running time. The electropherograms of fresh and frozen fish showed a good peak resolution between 10 and 15 min and between 16.5 and 21 min where 10 individual peaks were identified. Low resolutions were obtained for the eluted proteins between 15 and 15.6 min (zone 5–5′) and from 15.8–16.6 min (zone 7–7′). Both zones contained more than one peak. A total of 12 peaks or zones were identified in each electropherogram analyzed (Fig. 1).

Among the protein peaks, peak 1 was characteristic in all electropherograms and peak 3 showed the best resolution, sharpness and symmetry (Fig. 1). Peaks 1 and 3 were used to calculate relative migration time (RMT) and area percentage (AP), respectively (Shin-Shou, Shu-Shu, Hei-Wen, Deng-Fwu, Pei-Chin, & Shu-Chi, 1998).

### 3.2. CZE of SAR during frozen storage

Figs. 1–3 show the SAR separation in fresh and frozen fish at −18 °C and −30 °C stored up to 180 days. The resulting profiles of fresh and frozen fish showed common protein peaks and were clearly similar. A broadening of the peaks in some zones of the electropherograms took place as the storage progressed, slightly affecting the resolution of the CZE separation

Table 2
RMT and AP for each peak of SAR at −30 °C with corresponding standard deviations between individuals ($n = 3$, RMT relative migration time, AP percentage area of the peak)

| Time | Peak | 1 | 2 | 3 | 4 | 5 | 5′ | 6 | 7 | 7′ | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| T0 | RMT | 0.00 | 3.49 | 3.60 | 4.36 | 4.94 | 5.56 | 5.52 | 5.76 | 6.50 | 6.67 | 7.16 | 7.61 | 8.02 | 10.63 |
| T0 | SD | 0.00 | 0.03 | 0.02 | 0.02 | 0.07 | 0.28 | 0.08 | 0.09 | 0.09 | 0.10 | 0.12 | 0.13 | 0.09 | 0.09 |
| T0 | AP | 1.16 | 3.93 | 100 | 5.37 | 11.2 | – | 52.56 | 188 | – | 37.8 | 5.28 | 16.3 | 181 | 1.53 |
| T0 | SD | 0.55 | 0.91 | 0.00 | 1.02 | 1.20 | – | 5.00 | 2.25 | – | 1.75 | 1.59 | 7.36 | 7.68 | 0.23 |
| T30 | RMT | 0.00 | 3.52 | 3.63 | 4.49 | 5.02 | 5.45 | 5.60 | 5.80 | 6.60 | 6.78 | 7.49 | 7.72 | 8.17 | 11.25 |
| T30 | SD | 0.00 | 0.10 | 0.11 | 0.35 | 0.14 | 0.15 | 0.15 | 0.16 | 0.17 | 0.18 | 0.14 | 0.15 | 0.18 | 0.08 |
| T30 | AP | 1.56 | 3.68 | 100 | 5.02 | 7.93 | – | 69.4 | 177 | – | 34.6 | 7.50 | 6.34 | 214 | 2.19 |
| T30 | SD | 0.79 | 1.23 | 0.00 | 0.36 | 2.19 | – | 2.77 | 12.8 | – | 0.58 | 1.89 | 1.29 | 6.23 | 0.40 |
| T60 | RMT | 0.00 | 3.28 | 3.38 | 3.84 | 4.69 | 5.09 | 5.26 | 5.47 | 6.15 | 6.29 | 6.82 | 7.29 | 7.67 | 10.62 |
| T60 | SD | 0.00 | 0.16 | 0.17 | 0.52 | 0.21 | 0.27 | 0.27 | 0.27 | 0.32 | 0.34 | 0.34 | 0.37 | 0.36 | 0.38 |
| T60 | AP | 1.75 | 2.87 | 100 | 4.91 | 6.20 | – | 61.5 | 173 | – | 37.0 | 4.18 | 9.99 | 209 | 1.40 |
| T60 | SD | 1.07 | 2.49 | 0.00 | 1.78 | 2.79 | – | 0.55 | 4.74 | – | 4.66 | 2.18 | 3.97 | 12.9 | 0.40 |
| T90 | RMT | 0.00 | 3.25 | 3.34 | 4.32 | 4.69 | 5.03 | 5.18 | 5.42 | 6.08 | 6.20 | 6.68 | 7.15 | 7.54 | 0.11 |
| T90 | SD | 0.00 | 0.04 | 0.05 | 0.01 | 0.09 | 0.09 | 0.07 | 0.08 | 0.09 | 0.09 | 0.13 | 0.12 | 0.11 | 0.09 |
| T90 | AP | 2.08 | 1.92 | 100 | 4.51 | 5.37 | – | 52.4 | 173 | – | 27.0 | 5.76 | 6.21 | 158 | 1.86 |
| T90 | SD | 1.15 | 1.14 | 0.00 | 1.40 | 0.58 | – | 4.60 | 11.7 | – | 7.49 | 0.57 | 0.57 | 5.77 | 0.41 |
| T120 | RMT | 0.00 | 3.18 | 3.24 | 4.29 | 4.49 | 4.84 | 5.01 | 5.16 | 5.86 | 6.04 | 6.47 | 6.83 | 7.24 | 9.34 |
| T120 | SD | 0.00 | 0.04 | 0.06 | 0.07 | 0.09 | 0.09 | 0.08 | 0.08 | 0.10 | 0.12 | 0.11 | 0.19 | 0.12 | 0.12 |
| T120 | AP | 2.00 | 1.49 | 100 | 5.56 | 6.93 | – | 48.3 | 133 | – | 24.9 | 5.19 | 9.51 | 137 | 2.22 |
| T120 | SD | 1.28 | 1.47 | 0.00 | 1.35 | 0.84 | – | 8.54 | 0.91 | – | 7.53 | 0.73 | 1.23 | 7.00 | 0.84 |
| T150 | RMT | 0.00 | 3.13 | 3.22 | 4.30 | 4.51 | 4.83 | 5.00 | 5.20 | 5.84 | 5.96 | 6.41 | 6.88 | 7.25 | 9.99 |
| T150 | SD | 0.00 | 0.02 | 0.03 | 0.05 | 0.02 | 0.02 | 0.02 | 0.02 | 0.01 | 0.05 | 0.01 | 0.02 | 0.02 | 0.06 |
| T150 | AP | 2.36 | 2.06 | 100 | 7.87 | 7.03 | – | 48.1 | 141 | – | 22.9 | 5.87 | 17.0 | 120 | 1.56 |
| T150 | SD | 1.60 | 1.10 | 0.00 | 2.31 | 2.56 | – | 2.27 | 6.31 | – | 4.77 | 0.69 | 3.04 | 8.16 | 0.46 |
| T180 | RMT | 0.00 | 3.21 | 3.28 | 3.85 | 5.09 | 4.91 | 5.12 | 5.29 | 6.00 | 6.17 | 6.60 | 6.93 | 7.36 | 9.21 |
| T180 | SD | 0.00 | 0.05 | 0.01 | 0.10 | 1.11 | 0.01 | 0.02 | 0.03 | 0.02 | 0.02 | 0.03 | 0.03 | 0.07 | 0.10 |
| T180 | AP | 3.06 | 1.42 | 100 | 6.92 | 8.76 | – | 44.6 | 120.2 | – | 20.7 | 5.69 | 11.3 | 115 | 1.86 |
| T180 | SD | 2.19 | 1.22 | 0.00 | 1.53 | 1.74 | – | 8.67 | 7.29 | – | 5.45 | 0.48 | 3.13 | 8.95 | 0.77 |

Table 3
Protein concentration (SAR) in the water extract in frozen golden kinglip fish at −18 and −30 °C

| Time (days) | $n$ | Protein concentration (mg/ml) −18 °C | S.D. | Protein concentration (mg/ml) −30 °C | S.D. |
|---|---|---|---|---|---|
| 0 | 3 | 4.87 | 1.37 | 4.87 | 1.37 |
| 30 | 3 | 3.22 | 0.20 | 4.09 | 1.54 |
| 60 | 3 | 3.15 | 0.32 | 3.17 | 0.24 |
| 90 | 3 | 2.88 | 0.44 | 3.66 | 0.75 |
| 120 | 3 | 3.23 | 0.24 | 3.59 | 0.51 |
| 150 | 3 | 2.96 | 0.10 | 2.95 | 0.38 |
| 180 | 3 | 2.36 | 0.19 | 2.73 | 0.29 |

(zone 5–5′ and zone 7–7′). This broadening of some peaks could be attributed to protein degradation products which migrate in that zone. Tables 1 and 2 show RMT and AP values for each peak under the conditions studied. The variability of both parameters was established by analyzing three different specimens of golden kinglip fish species. Both fresh and frozen fish showed low standard deviation (S.D.) values for RMT and relatively high AP values. These values are similar to those reported by Piñeiro, Sotelo, Medina, Gallardo, and Pérez-Martin (1997) with raw and frozen gadoid fish and also to the low S.D. values for RMT reported by Gallardo et al. (1995), determined with profiles of eight flatfish species.

The relatively high S.D. values for AP might have been caused by differences in the amounts of SAR in the water extract (Piñeiro et al., 1997). However, the SAR content among different specimens was similar under the conditions studied (Table 3). The comparison of both frozen temperatures showed a decrease of SAR content with time, which was probably due to frozen storage time (LeBlanc & LeBlanc, 1989). A point of interest was a greater lowering in the SAR content found at −18 °C than at −30 °C in the first month of frozen storage, which indicates a major degradation of

Table 4
Variation of RMT of peaks of golden kinglip SAR during frozen storage at −18 °C (CV coefficient of variation)

| Time (days) | 0 | 30 | 60 | 90 | 120 | 150 | 180 | |
|---|---|---|---|---|---|---|---|---|
| Peak | RMT | RMT | RMT | RMT | RMT | RMT | RMT | CV-RMT |
| 1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 3.49 | 3.42 | 3.23 | 3.17 | 3.05 | 3.06 | 3.19 | 5.23 |
| 3 | 3.60 | 3.49 | 3.33 | 3.26 | 3.12 | 3.16 | 3.26 | 5.24 |
| 4 | 4.36 | 4.48 | 4.03 | 4.20 | 3.97 | 4.24 | 3.89 | 5.16 |
| 5 | 4.94 | 4.84 | 4.52 | 4.51 | 4.29 | 4.42 | 4.54 | 5.03 |
| 5′ | 5.56 | 5.27 | 4.98 | 4.94 | 4.65 | 4.72 | 4.92 | 4.18 |
| 6 | 5.52 | 5.43 | 5.18 | 5.11 | 4.81 | 4.89 | 5.08 | 5.06 |
| 7 | 5.76 | 5.61 | 5.41 | 5.32 | 4.97 | 5.08 | 5.28 | 5.20 |
| 7′ | 6.5 | 6.39 | 6.12 | 5.98 | 5.63 | 5.72 | 5.97 | 5.33 |
| 8 | 6.67 | 6.56 | 6.24 | 6.12 | 5.78 | 5.82 | 6.11 | 5.48 |
| 9 | 7.16 | 7.05 | 6.73 | 6.62 | 6.21 | 6.27 | 6.56 | 5.41 |
| 10 | 7.61 | 7.48 | 7.19 | 7.07 | 6.63 | 6.72 | 6.93 | 5.18 |
| 11 | 8.02 | 7.92 | 7.61 | 7.47 | 6.98 | 7.11 | 7.33 | 5.21 |
| 12 | 10.63 | 10.98 | 10.61 | 10.18 | 9.17 | 9.82 | 9.67 | 3.63 |

Table 5
Variation of RMT of peaks of golden kinglip SAR during frozen storage at −30 °C (CV coefficient of variation)

| Time (days) | 0 | 30 | 60 | 90 | 120 | 150 | 180 | |
|---|---|---|---|---|---|---|---|---|
| Peak | RMT | RMT | RMT | RMT | RMT | RMT | RMT | CV-RMT |
| 1 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 2 | 3.49 | 3.52 | 3.28 | 3.25 | 3.18 | 3.13 | 3.21 | 4.60 |
| 3 | 3.60 | 3.63 | 3.38 | 3.34 | 3.24 | 3.22 | 3.28 | 4.92 |
| 4 | 4.36 | 4.49 | 3.84 | 4.32 | 4.29 | 4.3 | 3.65 | 1.61 |
| 5 | 4.94 | 5.02 | 4.69 | 4.69 | 4.49 | 4.51 | 5.09 | 5.14 |
| 5′ | 5.56 | 5.45 | 5.09 | 5.03 | 4.84 | 4.83 | 4.91 | 5.73 |
| 6 | 5.52 | 5.60 | 5.26 | 5.18 | 5.01 | 5.00 | 5.12 | 4.51 |
| 7 | 5.76 | 5.80 | 5.47 | 5.42 | 5.16 | 5.2 | 5.29 | 4.68 |
| 7′ | 6.50 | 6.60 | 6.15 | 6.08 | 5.86 | 5.84 | 6.00 | 3.84 |
| 8 | 6.67 | 6.78 | 6.29 | 6.2 | 6.04 | 5.96 | 6.17 | 0.24 |
| 9 | 7.16 | 7.49 | 6.82 | 8.68 | 6.47 | 6.41 | 6.60 | 5.75 |
| 10 | 7.61 | 7.72 | 7.29 | 7.15 | 6.83 | 6.88 | 6.93 | 4.93 |
| 11 | 8.02 | 8.17 | 7.67 | 7.54 | 7.24 | 7.25 | 7.36 | 4.84 |
| 12 | 10.63 | 11.25 | 10.62 | 10.33 | 9.34 | 9.99 | 9.21 | 4.91 |

SAR versus temperature (LeBlanc et al., 1994). Relatively high frozen storage temperatures cause SAR denaturation, until SAR become unextractable (LeBlanc et al., 1989). However, the RMT of the peaks, under all conditions, showed minor variations, making them more useful for identification purposes compared with area determinations, which is in accordance with Piñeiro et al. (1997).

In order to test possible variations in protein profiles due to frozen storage, the reproducibility of the CZE procedure in terms of RMT was established. Tables 4 and 5 shows the coefficient of variation of RMT for golden kinglip fish at −18 and −30 °C, respectively. A significant variation in the AP values with respect to those for fresh fish was also observed for both conditions of frozen storage (Tables 6 and 7). This was probably a consequence of the denaturation of SAR. The denaturation is reflected by a loss of solubility, which becomes evident with lower AP values, mostly for the main peaks, such as peak 7, at both frozen temperatures. This could also mean both a change in resolution, which might be a direct consequence of protein structural change and an unfolding, which is also a manifestation of protein denaturation (Piñeiro et al., 1997). However, the variation coefficients of the RMT parameter were around 5% for most times and peaks (Tables 4 and 5), which makes the patterns obtained quite reproducible (Gallardo et al., 1995).

The results are also in accordance with Ragnarsson and Regenstein (1989) who reported that most electrophoretic techniques, applied in fish muscle after frozen storage, have not shown any major difference among muscle proteins, except for a general loss in solubility.

In conclusion, CZE seems to be independent of any protein damage that might occur during frozen storage, since no significant RMT variations were detected for

Table 6
Variation of AP of peaks of golden kinglip SAR during frozen storage at −18 °C (CV coefficient of variation)

| Time (days) | 0 | 30 | 60 | 90 | 120 | 150 | 180 | |
|---|---|---|---|---|---|---|---|---|
| Peak | AP | AP | AP | AP | AP | AP | AP | CV-AP |
| 1 | 1.16 | 1.72 | 1.70 | 2.20 | 2.08 | 2.96 | 3.21 | 37.46 |
| 2 | 3.93 | 2.40 | 4.10 | 1.56 | 1.66 | 1.90 | 2.10 | 47.79 |
| 3 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 0.00 |
| 4 | 5.37 | 4.19 | 4.66 | 5.09 | 3.54 | 7.26 | 5.27 | 24.2 |
| 5 | 11.2 | 6.90 | 6.43 | 9.33 | 4.57 | 6.05 | 7.35 | 32.1 |
| 6 | 60.5 | 60.7 | 58.1 | 55.5 | 57.1 | 50.6 | 56.0 | 6.07 |
| 7 | 216 | 180 | 185 | 164 | 143 | 148 | 134 | 17.7 |
| 8 | 32.5 | 24.0 | 28.2 | 27.6 | 26.8 | 24.1 | 22.6 | 12.8 |
| 9 | 5.28 | 6.82 | 4.91 | 4.62 | 4.41 | 5.88 | 7.09 | 19.6 |
| 10 | 16.3 | 12.9 | 14.3 | 14.8 | 9.93 | 24.7 | 20.1 | 32.6 |
| 11 | 192 | 198 | 195 | 149 | 141 | 108 | 105 | 27.7 |
| 12 | 1.53 | 1.10 | 1.71 | 1.68 | 1.91 | 2.02 | 2.23 | 22.0 |

Table 7
Variation of AP of peaks of golden kinglip SAR during frozen storage at −30 °C (CV coefficient of variation)

| Time (days) | 0 | 30 | 60 | 90 | 120 | 150 | 180 | |
|---|---|---|---|---|---|---|---|---|
| Peak | AP | AP | AP | AP | AP | AP | AP | CV-AP |
| 1 | 1.16 | 1.56 | 1.75 | 2.08 | 2.00 | 2.36 | 3.06 | 35.3 |
| 2 | 3.93 | 3.68 | 2.87 | 1.92 | 1.49 | 2.06 | 1.42 | 43.5 |
| 3 | 100 | 100 | 100 | 100 | 100 | 101 | 101 | 0.00 |
| 4 | 5.37 | 5.02 | 4.91 | 4.51 | 5.56 | 7.87 | 6.92 | 22.6 |
| 5 | 11.2 | 7.93 | 6.2 | 5.37 | 6.93 | 7.03 | 8.76 | 27.2 |
| 6 | 60.5 | 69.4 | 47.4 | 52.4 | 48.3 | 48.1 | 44.6 | 16.6 |
| 7 | 216 | 200 | 195 | 162 | 146 | 154 | 130 | 18.3 |
| 8 | 32.5 | 28.9 | 32.0 | 27.0 | 24.9 | 22.9 | 20.7 | 16.2 |
| 9 | 5.28 | 7.50 | 4.18 | 5.76 | 5.19 | 5.67 | 5.69 | 18.3 |
| 10 | 16.3 | 6.34 | 9.99 | 6.21 | 9.51 | 17.0 | 11.3 | 46.4 |
| 11 | 192 | 221 | 205 | 158 | 137 | 129 | 115 | 24.6 |
| 12 | 1.53 | 2.19 | 1.40 | 1.86 | 2.22 | 1.56 | 1.86 | 18.6 |

fish stored at −18 or −30 °C up to 180 days. In addition, identification made on the basis of RMT was successful, showing that CZE is able to characterize golden kinglip fish in frozen state for a long time in a rapid, simple and reproducible manner.

## Acknowledgements

This work was supported by funds from Facultad de Ciencias Químicas y Farmacéuticas. Universidad de Chile.

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