Food Chemistry 120 (2010) 304–307

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Capillary electrophoresis for bovine and ostrich meat characterisation

Belinda Vallejo-Cordoba *, Roberto Rodríguez-Ramírez, Aarón F. González-Córdova

Laboratorio de Calidad y Autenticidad de los Alimentos, Centro de Investigación en Alimentación y Desarrollo, A.C. (CIAD), Carretera a La Victoria Km. 0.6, Hermosillo, Sonora 83000, Mexico

ARTICLE INFO

Article history:
Received 31 December 2008
Received in revised form 18 September 2009
Accepted 22 September 2009

Keywords:
Bovine and ostrich meat
Capillary gel electrophoresis
Species differentiation

ABSTRACT

The objective of this study was to characterise, compare and quantify the water soluble protein (WSP) and salt soluble protein (SSP) fractions from bovine and ostrich muscle by using sodium dodecyl sulphate polymer-filled capillary gel electrophoresis (CE-SDS). Samples were raw ostrich leg and eye round beef collected 24 and 48 h, respectively, after sacrifice from local slaughter houses. WSP were extracted with cold double distilled deionized water and SSP with 0.6 M NaCl/0.01 M phosphate buffer pH 6 with 0.5% polyphosphates. Separation of WSP and SSP extracts was achieved by CE-SDS. Quantitative data for individual proteins was generated by constructing a calibration curve using bovine serum albumin (BSA) as a standard. The WSP profiles showed differences for bovine and ostrich meat, both qualitatively and quantitatively and could be employed for species differentiation. Quantitative data derived for WSP and SSP from bovine and ostrich muscle showed significant differences among individual proteins. A comparison of protein profiles form ostrich and bovine meat should be useful for meat species differentiation and muscle characterisation for establishing relations to meat quality.

1. Introduction

Meat is the edible animal flesh used as food after completion of a series of biochemical and biophysical changes initiated in the muscles after the death of the animal. Proteins, the most important functional components of the muscle, confer many of the desirable physicochemical and sensory attributes of muscle foods (Xiong, 1997). Muscle proteins comprise 15–22% of the total muscle weight and can be divided into three major groups on the basis of solubility characteristics: sarcoplasmic proteins (water soluble), myofibrillar proteins (salt soluble), and stroma proteins (insoluble). Of the three groups, myofibrillar proteins, which comprise 55–60% of total protein in muscle, play the most critical role during meat processing as they are responsible for the formation of thermally induced cohesive structures and the firm texture of meat products. These functional properties are the major factors contributing to palatability or sensory perception of processed meat products (Xiong, 1997).

In recent years the markets for ostrich meat are growing as a good alternative for other meats because it shows low fat and cholesterol content and is considered a good alternative for other meats (Majewska et al., 2009; Paleari et al., 1998; Sales, 1996, 1998; Viljoen, Hoffman, & Brand, 2005). Relative to beef, ostrich meat is characterised by a high ultimate pH, low collagen solubility, high pigment content, comparable cooking loss, dark visual appearance, comparable sensory tenderness and high polyunsaturated fatty acid content (C18:2ω6, C18:3ω3, C20:4ω6) (Girolami et al., 2003; Sales, 1996, 1998; Walter, Luann Soliah, & Dovalee Dorsett, 2000). Except for these technical reports addressing specific subjects, no literature is available on protein characterisation of ostrich muscle.

While the potential of capillary electrophoresis in food analysis has been recognised (Vallejo-Cordoba & Vargas-Martínez, 2008), its application to study muscle proteins is still very limited (Vallejo-Cordoba, González-Córdova, Mazorra-Manzano, & Rodríguez-Ramírez, 2005). Capillary free zone electrophoresis has been employed to separate fish muscle sarcoplasmic proteins (LeBlanc, Singh, & LeBlanc, 1994) and to differentiate flatfish species (Gallardo, Sotelo, Piñeiro, & Pérez-Martin, 1995). A CE-SDS procedure was developed in our laboratory that allowed the detection of qualitative and quantitative differences in sarcoplasmic protein in raw beef, pork and turkey meat (Cota-Rivas & Vallejo-Cordoba, 1997). Although the applicability of this methodology for meat species differentiation was suggested, the need for the analysis of multiple samples was recommended. Further work addressed the same problem by applying linear discriminant analysis in the interpretation of CE-SDS muscle protein profiles (Vallejo-Cordoba & Cota-Rivas, 1998).

Electrophoresis separations of muscle proteins in polyacrylamide gels in the presence of sodium dodecyl sulphate (SDS) and 2-mercaptoethanol is widely used for the qualitative analysis of proteins and was shown to be a reliable method for molecular weight determination (Claeys, Uytterhaegen, Buts, & Demmeyer,

* Corresponding author. Fax: +52 662 289 2400x303.
E-mail address: vallejo@ciad.mx (B. Vallejo-Cordoba).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2009.09.080

1995). Nevertheless, the quantification of the separated muscle proteins remained a complex issue. As a result, a semi-quantitative method for the determination of muscle proteins by densitometry scanning of stained SDS–polyacrylamide gels, using BSA as an internal standard was developed (Claeys et al., 1995). However, there is a need for quantitative methods for the determination of muscle protein concentrations that would allow establishing their relationship to meat quality.

The aims of this study were to characterise, compare and quantify the water soluble and salt soluble fractions of bovine and ostrich muscle by using CE-SDS.

2. Materials and methods

2.1. Samples

Fresh meats used were raw ostrich leg and eye round beef collected 24 and 48 h, respectively, after sacrifice from local slaughter houses (Hermosillo, Sonora, Mexico). Five samples of beef and five samples of ostrich were collected in two different occasions (n = 10 each) were transported to the laboratory under refrigeration.

2.2. Reagents

CE-SDS protein run and sample buffers, 2-mercaptoethanol and the broad-range molecular weight standards were purchased from Bio-Rad Laboratories (Hercules, CA); sodium hydroxide and sodium chloride were from Merck (Naucalpan, Mexico). Benzoic acid, bovine serum albumin (A 6003) and myosin (M 6643) were from Sigma Chemical Co. (St. Louis, MO); hydrochloric acid was from E.M. Science (Gibbstown, NJ); and polyphosphates were from Griffith (Monterrey, Mexico). Double distilled deionized water was used. All samples were filtered through acrylic prefilters and 0.2 μm nylon membrane from Millipore Corp. (Bedford, MA).

2.3. CE-SDS muscle protein characterisation

For quantitative analysis of meat protein profiles, samples were analysed in duplicates by a CE-SDS technique previously reported (Cota-Rivas & Vallejo-Cordoba, 1997). Briefly, meats (10 g) were mixed with 30 mL of water (bidistilled deionized) or saline buffer (0.6 M NaCl/0.01 M phosphate buffer, pH 6.0, 0.5% polyphosphates), blended at the highest speed with a LB10G Waring Blender (Waring Laboratory Science, Torrington, CT) and centrifuged at 7740 or 27200g for 15 min (4 °C) using a JA-21 centrifuge with a JA-20 rotor (Beckman Instruments, Fullerton, CA). Samples (60 μL) of the filtered supernatant, sample buffer (130 μL) containing benzoic acid as internal standard (0.7 mg/mL) and mercaptoethanol (60 μL of a 16.6% solution) were mixed in polypropylene micro test tube. The microtest tubes were then placed in boiling water for 10 min and then cooled to 4 °C. Filtered samples with a total protein of 1.5–3.5% were loaded by pressure injection at 50 psi/s for 8 s. The separation was carried out with CE-SDS run buffer (Bio-Rad) in a bare silica capillary (18 cm, 50 μm id) at 20 °C mounted in a BioFocus 3000 Capillary Electrophoresis System (Bio-Rad Laboratories, Hercules, CA). A constant voltage of 10 kV with reversed polarity was used. Quantitative data for WSP extracts and SSP extracts were derived by using normalised peak areas (area/migration time) obtained with detection at 214 nm. Muscle total protein was determined by Kjeldahl (AOAC, 1997).

2.4. Calibration curve

Bovine serum albumin was dissolved in double distilled deionized water at 5, 7, 5, 10 and 12.5 mg/mL and prepared as meat sample extracts for CE-SDS analysis. BSA protein solutions and meat sample extracts were analysed in duplicate, and normalised peak areas were obtained for each protein.

2.5. Statistical analysis

Linear regression analysis of the BSA calibration curve and differences between mean protein concentrations for beef and ostrich extracts with a pairwise comparison of means (t-test) were performed on a Systat program (Systat, Inc., Evanston, IL, USA).

3. Results and discussion

The electropherograms of the water soluble proteins showed differences for bovine and ostrich meat, both qualitatively and quantitatively (Fig. 1). It can be seen that although most peaks were common to both species, peak 3 was not detected in beef. The presence of bovine serum albumin (66.3 kDa), the most abundant of the sarcoplasmic proteins in ostrich meat, was observed and identified (peak 8) by using broad-range molecular weight

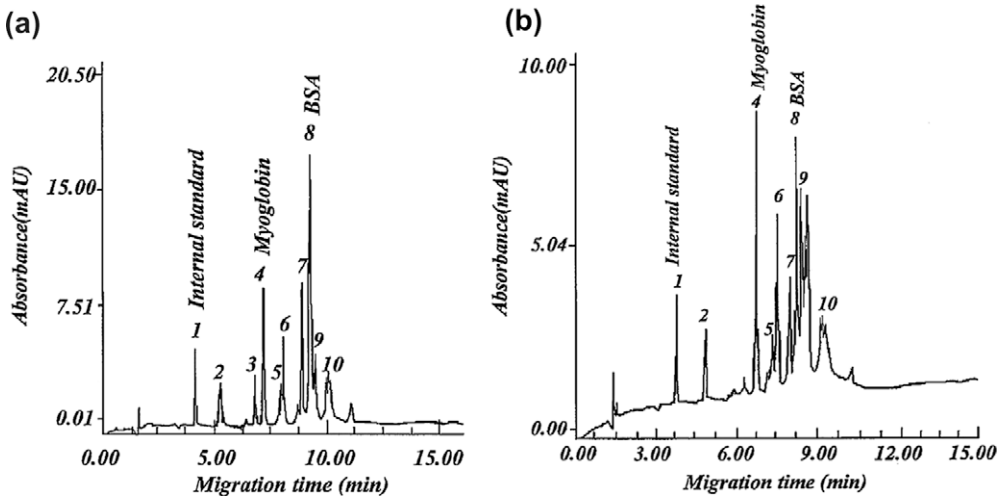

Fig. 1. CE-SDS electropherograms of water soluble proteins obtained by CE-SDS (10 kV, 50 psi/s, UV detection at 214 mm). Peaks 4 and 8 were identified as myoglobin and bovine serum albumin: (a) ostrich meat and (b) bovine meat.