ELECTROPHORETIC CHARACTERIZATION OF THE MUSCLE PROTEINS OF POULTRY

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Fragments of poultry breast muscle (broiler chicken and turkey) were subjected to four electrophoretic techniques: Native Gel (Polyacrylamide) Electrophoresis, Non-Denaturing SDS-PAGE, CELM-GEL® General Agarose, and Isoelectrofocalization in Phast Gel, as a means to identify the standardization of the electrophoretic profile of the species under study, by allying electrophoretic methods with densitometry, what enables the evaluation of possible losses on the protein fractions being handled. The results demonstrate specific patterns of muscle protein for each species and are useful in the identification of muscle proteins of chickens and turkeys. Besides, densitometry showed individual quantitative variations that, though identified losses, did not threaten the specific standardization of the electropherograms.

Key Words – SDS-PAGE, Densitometry, muscle protein.

I. INTRODUCTION

The feeding habits throughout human evolution acquired great importance, for they are essential sources of natural nutrients. Due to the growing demand of people for this kind of food, it is noteworthy that poultry is the most consumed meat in the world and, in Brazil, the biggest meat production is of broiler chickens, with over one million tons in relation to beef, which comes in second place (UBABEF, 2011; IBGE, 2011) [1,2]. In relation to other species of commercial interest, Pulici et al. (2008) [3] shows that 36.9 million turkeys were slaughtered in 2005, representing a 5.61% growth in relation to 2004. Besides, the production reached 359.2 tons, which means a 14.21% growth and, in 2010, exports totalized nearly 160 thousand tons of meat, almost 50% of which was in industrialized goods, and the main consuming market was Europe (UBABEF, 2011) [1]. Among the expected characteristics for the identification standard of meat products, sensorial characteristics like softness, succulence and smell are the most required by the consumer, and it so happens that these are directly linked to the protein constitution of the product (TATUM *et al*., 2000) [4]. Their high complexity is due to the fact that they are constituted by different sequences of amino acids, which, by the way, may or may not be modified, besides showing a wide range of size (varying from a few kDa to some MDa) and structures (NEVES, 2010) [5]. Bearing this in mind, it is now possible to mention evaluation and quantification techniques – electrophoresis and densitometry (MAFRA, *et al*., 2008) [6]. Electrophoresis by polyacrylamide gel enables the obtainment of a specific and standard pattern for different species of animals, a pattern that is not altered by age, breeding systems, slaughter methodologies and post-mortem periods, in terms of large protein fractions (DIERCKX et al., 2004) [7]. This paper aimed at identifying the standardization of the muscle protein electrophoretic profile of poultry species, by using different electrophoretic methods and, in an alliance with densitometry, evaluating the possible losses in the fractions found.

II. MATERIALS AND METHODS

For sampling purposes, 30 breast muscle fragments of poultry, ten of each species under study (broiler chickens and turkeys) were collected, and the animals were slaughtered in São Paulo and Paraná slaughterhouses. As for the fragments of chicken and turkey, complete pieces of their breasts were removed, and only then did the fragments get collected. All samples were

collected in the sequence of slaughter, with animals of the same lot and, therefore, the same breeding system for each of the species being studied. After being taken, the samples were stored in individual packs, tagged and moved to freezers with the set point at -18°C. The alterations caused by the animal's post-mortem were not taken into account, since the protein pieces in study would not be affected (DIERCKX *et al*., 2004) [7]. For protein extraction, 500 mg-samples of muscle fragments conserved in liquid nitrogen were used. After freezing, the muscles were sliced and macerated in a mortar containing 2 ml of distilled and deionized distilled water, according to the technique described by Lemos; Moraes (1992) [8]. The resulting samples were centrifuged at the temperature of 4°C, with 7500 G for 15 minutes. The precipitate was discarded and the supernatant was diluted in a solution for application at a proportion of 1:3 (v/v) and frozen at – 80°C, and later diluted and used in the electrophoretic run. Then, the mini-PROTEAN 3 set (BIORAD®) was applied for the performance of the PAGE electrophoresis, following the manufacturer's instructions and the technique suggested by Hames; Rickwood (1990) and altered by Ramos (1992) [9]. Each sampling well received an aliquot sample of 13 µl, connecting the vat to the source follower, maintaining the following protocol: a 100 V initial current for 10 minutes before a 300 V current for 150 minutes, the amperage being kept free. This same protocol was applied on the non-denaturing SDS-PAGE, with the addition of SDS in the preparation of the separating gel. The sampling quantities that were utilized, as well as the source follower protocol, were kept the same. A layer of agarose was used for the agarose gel electrophoresis, following the specifications of the manufacturer (CELM-GEL), adding 90 ml of the TRIS buffer, pH at 9.5, cooled, and samples of 1 µl were placed inside each well, on the following source follower protocol: a 100 V current for 20 minutes, with free amperage. For the use of the isoelectrofocalization mini-gel sets (pH gradient 3-9), the gels were immediately placed in the equipment, and samples of 3 µl were applied on to the application board, so that the combs were later used (12 positions, the lateral positions being discarded). In this case – by capillarity – each comb collected aliquots of 0.3 µl. The chosen protocol of the PHASTSYSTEM equipment (PhastGel® GE HEALTH-CARE) was as follows: a 100 V charge until samples got adhered to the gel, then a 2000 V electric potential difference for the separation of the protein fractions, for 20 minutes. Gel staining was carried by immersion in the Coomasie Brillant Blue dye in a solution of acetic acid, in accordance with manufacturer instructions, during 4 hours for the PAGE and SDS-PAGE techniques. After staining, the gel was stored in a Coomasie destaining solution (water/methane/acetic acid at a proportion of 1:3:6, v/v), until the protein bands were developed. As for the agarose technique, staining was carried by employing black starch at 0.1% in a 5% acetic acid solution, and the destaining process was carried in a 5% acetic acid solution until the full development of the protein bars. The mini-gel sets were subjected to staining and destaining in the device itself (PHASTSYSTEM), in accordance with recommendations from the manufacturer. The capture of gel images was carried by means of a photo-documentation equipment (Image Máster VDS Pharmacia), with the identification of the values for relative mobility, molecular weights and isoeletrical points. For the quantification of the values, the photodocumentation software carried the densitometric analyses, making the quantitative evaluation of each of the protein bands, which were separated by the different electrophoretic techniques.

III. RESULTS AND DISCUSSION

Considering the agarose gel electrophoresis, six protein bands were observed in the chicken samples and five in turkey samples. By evaluating the relative mobility values, we discovered that only the r1 band (0.110) was found in the samples of chicken and turkey (Figure 1), what was also demonstrated through the densitometry results. The rest of the bands did not show any relations among the tested species, just as no similarities among the gels were found. The descriptive statistics demonstrated that the technique is both accurate and replicable, as the values for standard deviation, coefficient of variation and the confidence interval of the relative mobility figures were all extremely low. As for the results of the densitometric analysis, a great homogeneity among the tested species was found, what thus guarantees the existence of a pattern that

corresponds to the electrophoretic profile that was obtained and characterized. The achieved results demonstrate that the obtainment of a protein pattern for the species studied in this paper can be reached by employing this technique.

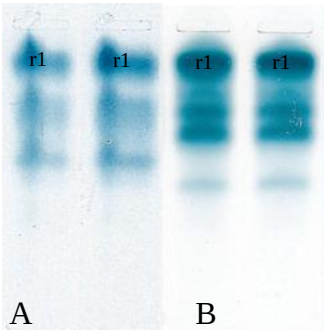

Figure 1. Agarose Gel Electrophoresis of hydrosoluble muscle proteins of the breasts of broiler chickens (A) and turkeys (B).

Bearing the native gel (polyacrylamide) electrophoresis technique as a reference, six protein bands were observed in the chicken samples and eight in the turkey samples. Using this technique showed a higher protein fractioning than the one that results from the agarose gels, and no relation between the occurrences of protein bands among the studied species was found (Figure 2). This fact was noted by analyzing the relative mobility values of the bands, using the mentioned technique. We also noted that chicken samples, just as turkey samples, presented very low values for standard deviations and coefficient of variation, what accounts for the replicability and accuracy of the technique. The same can be described as of the values concerning densitometry, the individual differences of which, though they actually have been found, showed a minimal variation, just as demonstrated through the descriptive statistics. The results herein obtained proves the employability of this technique for the achievement of a protein pattern to the studied species, with a higher diversity of protein bands in relation to the technique previously mentioned.

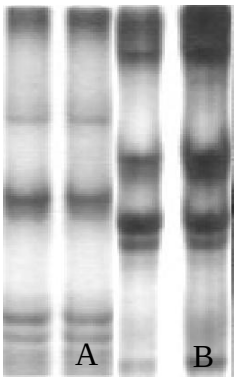

Figure 2. Electrophoresis of hydrosoluble muscle proteins in a polyacrylamide gel at a concentration of 10% in the separating gel, in a non-continuous systems of alkali buffers of broiler chickens (A) and turkeys (B).

The results obtained with the native gel (polyacrylamide) electrophoresis technique in the presence of a non-reducing and non-denaturing SDS indicated the presence of three protein bands in chicken samples and six in turkey samples. That is why, in this sense, we noted the non-occurrence of coincident bands among the tested species (Figure 3), just as shown by the relative mobility data and by the low values of standard deviation and coefficient of variation. Despite the reduction in the number of revealed bands, the formation of an individual electrophoretic and non-coincidental pattern among the studied species was also observed, what enables one to use this technique in telling one species from another.

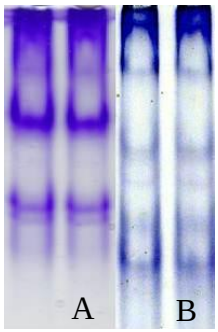

Figure 3. Electrophoresis of hydrosoluble muscle proteins from the breast of broiler chickens (A) and turkeys (B) in polyacrylamide gel at a concentration of 10% in the separating gel, in the presence of sodium dodecyl sulfate (SDS), non-denaturing and non-reducing, in a discontinuous system of alkali buffers.

And, at last, the results obtained in the isoelectrofocalization technique demonstrated a higher interspecific variation for muscle proteins.

Six protein fractions were detected in chicken samples, eleven fractions in turkey samples and twenty fractions in the ostrich samples. By analyzing the results for relative mobility it was possible to note that band r6 in chicken samples coincided with band r9 of turkey samples. (Figure 4). Coincidences apart, the electrophoretic patterns obtained for the different species were quite different. But similarly, the low values for standard deviations and coefficient of variation point in the direction of the replicability of the results obtained with this technique, constituting an individual and standardized electrophoretic pattern for each tested species.

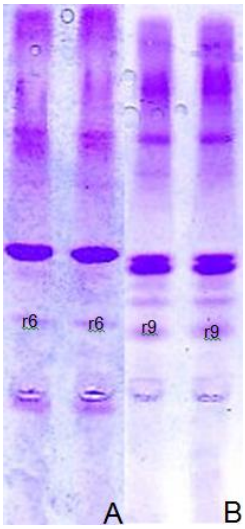

Figure 4. Isoelectrofocalization of hydrosoluble muscle proteins of the breasts of broiler chickens (A) and turkeys (B).

IV. CONCLUSION

The electrophoretic techniques employed revealed electropherograms of muscle proteins that, either bearing a bigger or smaller fractioning, resulted in specific patterns of muscle proteins for each species, and are useful in identifying the muscle tissues of chickens and turkeys. Densitometry demonstrated the occurrence of individual quantitative variations which – probably due to protein losses during manipulation or to alterations that concern the very individual gene expression – did not threaten the specific standardization of the electropherograms.

REFERENCES

1. UNIÃO BRASILEIRA DE AVICULTURA (UBABEF, 2011). Relatório anual 2011: Anuário da Avicultura Brasileira. 72p. In: <http://www.abef.com.br/ubabef/exibenoticiaubabef.php?notcodigo=2761>.
2. INSTITUTO BRASILEIRO DE GEOGRAFIA E ESTATÍSTICA (IBGE, 2011). Indicadores IBGE: Estatística da Produção Pecuária. Comissão Especial de Planejamento, Controle e Avaliação das Estatísticas Agropecuárias (CEPAGRO), Brasília, DF. 32p.
3. Pulici, R., Alves, F. R., Gameiro, A. H (2008). Aceitação e segmentação do mercado de produtos derivados de peru. In: XLVI Congresso da Sociedade Brasileira de Economia, Administração e Sociologia Rural. Oral presentation.
4. Tatum, J. D.; Smith, G. C.; Belk, K. E. (2000). New approaches for improving tenderness, quality, and consistency of beef. Journal of Animal Science, v.77, p. 1-10.
5. Neves, R. C. F. (2010). Desenvolvimento de metodologias analíticas para estudo metaloproteômico do cobre em plasma, tecido muscular e hepático da Tilápia do Nilo, Thesis, . 115p.
6. Mafra, I., Ferreira, I. M. P. L. V. O., Oliveira, M. B. P. P (2008). Food authentication by PCR-based methods. European food research and technology, v. 227, Issue 3, p. 649-665.
7. Dierckx, S.M.; Ramos, P.R.R.; Bortolozzi, J (2004). Identificação das proteínas musculares de suínos submetidas à eletroforese em gel de poliacrilamida com Sodium Dodecil Sulfato (SDS). Archivos Latinoamericanos de Producción Animal, v.12, n.1, p.8-11.
8. Lemos, A. L. S. C., Moraes, M. A. C. (1992) Identificação eletroforética de peixes de água doce (Família *Pimelodidae*) de valor comercial**. Alimentos e Nutrição – Brazilian Journal of Food and Nutrition**, v.4, p.57-63.
9. Ramos, P. R. R. (1992). **Polimorfismo bioquímico de proteínas séricas do leite de vacas da raça holandês, puras por cruzamento, variedade malhada de preto**. Thesis. 131f.