PE4.07 SDS-PAGE electrophoretic pattern of pork loin enhanced with non-meat proteins 25.00

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***Abstract*— Brazilian meat regulations allow the use of non-meat extenders in certain meat products. Thus, the need for controlling the use of these ingredients is evident, in order to avoid frauds. In the present work, sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS-PAGE) has been applied to pork enhanced with soy protein isolate (SPI), whey protein concentrate (WPC) and sodium tripolyphosphate (STTP), in the presence of positive and negative controls through two different methods of protein extraction: urea 6M / Tris-HCl-SDS-mercaptoethanol (ME). Electrophoresis has revealed typical whole-muscle profiles, with the soy proteins bands overlapping with meat proteins. Whey proteins, however, have arisen without interference with meat proteins, showing that with SDS-PAGE electrophoresis it has been possible to detect the addition of this extender in enhanced pork.**

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***Index Terms*—meat products, enhancement, non-meat proteins, electrophoresis.**

I. INTRODUCTION

Since the 70's, electrophoresis has been applied to foods in order to avoid frauds and to inspect the use of proteins strange to their normal composition.

In many countries the processing of enhanced meats has been continuously increasing, and their several benefits for both industry and consumers have been reported by many authors [3, 8].

Brazilian meat inspection regulations allow the use of non-meat proteins from 2% (in cooked hams) to 4% (in hamburgers and cooked sausages) in several processed meat products. However, the addition of non-meat proteins to meat products may cause health problems, mainly to allergic individuals which can be affected by the ingestion of the allergen. Thereby, for being subjected to legal limitations, the addition of non-meat proteins needs to be controlled [1].

Pork enhancement is not yet allowed by federal authority in Brazil, and fraudulent injected meats without proper labeling have been marketed in several regions of the country. Furthermore, Brazilian additives industries have been implemented technologies to incite this fraud, offering a wide range of binders and extenders to be irregularly injected into meat.

In this work, we evaluated the application of SDS-PAGE to control the use of SPI and WPC as non-meat-proteins to enhance pork loins.

II. MATERIALS AND METHODS

A. Samples

Five whole fresh pork loins (24 h post-mortem, pH 5.6-5.9) were selected to each treatment and then cut into three sections each. The loin sections were randomly assigned for enhancement and pumped to 115% of original weight with a brine solution using a multi-needle brine injector. Six brine solutions were formulated: 5% salt (treatment B); 5% salt and 3% STTP (treatment C); 5% salt and 10% SPI (treatment D), 5% salt and 10% WPC (treatment E), 5% salt, 10% SPI and 3% STTP (treatment F) and 5% salt, 10% WPC and 3% STTP (treatment G) for comparison with non-enhanced control loins (treatment A). Each specific treatment marinade was manufactured by adding in sequence, the appropriate amount of cold water (4°C) and non-meat ingredients, until their complete dissolution. Treatment marinades were randomly assigned to 15 loin sections each. The injection machine was thoroughly cleaned between each treatment. After injection, loins were vacuum packaged and held for 72 h at 2°C to allow for equilibration of the injected solution throughout the loin. Loins were then sliced into chops, vacuum packaged, stored at 4°C and analyzed within 30 days. Final concentrations in the injected meat were 5 g of salt, 0.45 g of STTP and 1.5 g of non-meat proteins per 100 g of meat, depending on the brine, assuming all the injected ingredients are retained. The protein content in the non-meat proteins used for brine formulations were 75% w/w (WPC) and 80% w/w (SPI).

B. Extraction with urea 6 M

A 10 g portion of each ground meat sample was blended with 30 ml of a 6 M urea solution for 2 minutes. The mixture was then heated to 70°C for 2 minutes. This extract was centrifuged at 3,000 *g* for 15 minutes at 4°C and then a 400 µl sample of supernatant was mixed with 200 µl sample buffer [5]. From this mixture, a 10 µl from each treatment was taken for electrophoresis.

C. Extraction with Tris-HCl-SDS-Mercaptoethanol

A 10 g portion of each ground meat sample was blended with a solution containing 0.0625 M Tris-HCl (pH 6.8), 3% SDS and 1% β-mercaptoethanol [7]. This extract was centrifuged at 3,000 *g* for 15 minutes at 4°C. Supernatants were diluted at 1:6 for electrophoresis. Protein concentration was determined by the Bradford method.

D. Positive controls

Non-meat proteins positive controls were run with the enhanced samples for electrophoresis by mixing 600 µl of meat extract (non-enhanced loin) with 100 µl of SPI or WPC, with 0.01 and 0.1 g/mg concentrations, respectively.

E. Electrophoresis

SDS-PAGE was performed with a 12% acrylamide resolving gel stacked with a 4% acrylamide gel [5]. Electrophoresis was conducted with a 100 v constant current per gel. In order to estimate the molecular weights (M_w) of protein bands, protein standards (19.445-211.240 kDa, Sigma Chemical Co., St. Luis, MO, USA) were electrophoresed together with pork muscle samples. The gels were stained in a solution of 1% Coomassie blue R250, under continuous gentle agitation. M_w of individual proteins were determined from the regression line of the protein migration distance versus log (M_w).

III. RESULTS AND DISCUSSION

Fig. 1 shows the typical whole-muscle electropherogram obtained with the urea-extraction of proteins of pork samples and the controls ran together with them. It fits with pork myofibrillar patterns described by other authors [6, 9], showing the proteins characteristic patterns so that mixtures of meat and whey proteins can be detected. The most distinct bands on meat samples are myosin heavy chain (207 kDa) and actin (46 kDa).

Fig. 2 shows the electrophoretic profile obtained with the Tris-HCl-SDS-ME extraction. As expected, myoglobin bands (17 kDa) are discrete and overlapped by β-lactoglobulin (18 kDa) in treatments 5 and 7 (lanes 6 and 8). This extraction protocol allowed a clearer visualization of the bands for both meat samples and controls, compared to extraction with urea 6 M.

Contrary to the positive controls, where at least one band discriminates the meat extracts from the extenders, loin samples patterns presented an interference of proteins from soy with those from meat on both extraction protocols. Thus, soy could not be detected. Since non-meat proteins on pork samples are expected to be about 1.5% in the present study, these patterns are contrary to several reports which have been detected soy in meat at levels down to 0.5% [1]. Although electrophoresis has been largely applied for the detection of soy in meat products, this method can yield crowded electropherograms, making it difficult to detect the presence of bands originating from added soy proteins [2]. Furthermore, it is extremely difficult to detect the presence of bands originating from added non-meat proteins because these bands are always of minor intensity compared to bands originating from the meat themselves [4].

On the other hand, with detailed examination, whey proteins can be noticed in enhanced samples on both urea and Tris-HCl-SDS-ME extractions, although they were more evident on the latter. On both Fig. 1 and Fig. 2, arrows point to whey protein bands that can be detected in the enhanced pork samples of the present study, corresponding to α-lactalbumin (13 kDa) and β-lactoglobulin (19 kDa), respectively.

IV. CONCLUSION

SDS-PAGE was efficient enough to detect the whey proteins added to the enhanced pork samples, but the soy was not detected by any of the extraction protocols. Thus, further research is still needed in order to control the use of non-meat proteins in meat products by means of electrophoresis, mainly the soybean proteins, which are among the most used extenders by meat industry in Brazil.

REFERENCES

[1] Belloque, J., Garcia, M.C., Torre, M. & Marina, M.L. (2002). Analysis of soyabean proteins in meat products: a review. Critical Review in Food Science and Nutrition, 42(5), 507-532.

[2] Castro-Rubio, F., Garcia, M.C., Rodriguez, R. & Marina, M.L. (2005). Simple and inexpensive method for the reliable determination of soybean proteins in heat-processed meat products: an alternative to the AOAC official method. Journal of Agricultural and Food Chemistry, 53(2), 220-226.

[3] Hayes, J.E., Desmond, D.M., Troy, D.J., Buckley, D.J. & Mehra, R. (2006). The effect of enhancement with salt, phosphate and milk proteins on the physical and sensory properties of pork loin. Meat Science, 72(3), 380-286.

[4] Janssen, F.W., Voortman, G., & De Baaij, J.A. (1987) Detection of wheat gluten, whey protein, casein, ovalbumin, and soy protein in heated meat products by electrophoresis, blotting, and immunoperoxidase staining. Journal of Agricultural and Food Chemistry, 35(4), 563-567.

[5] Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head bacteriophage T4. Nature, 227, 680-685.

[6] Larrea, V., Hernando, I., Quiles, A., Lluch, M.A., & Pérez-Munuera, I. (2006). Changes in proteins during Teruel dry-cured ham processing. Meat Science, 74(3), 586-593.

[7] Lee, Y.B., Rickansrud, D.A., Hacberg, E.C., & Forsythe, R.H. (1976). Detection of various nonmeat extenders in meat products. Journal of Food Science, 41(3), 589-593.

[8] Sheard, P.R., & Tali, A. (2004). Injection of salt, tripolyphosphate and bicarbonate marinade solutions to improve the yield and tenderness of cooked pork loin. Meat Science, 68(2), 305-311.

[9] Xiong, Y.L., Gower, M.J., Li, C., Elmore, C.A., Cromwell, G.L., & Lindemann, M.D. (2006). Effect of dietary ropamine on tenderness and postmortem protein degradation of pork muscle. Meat Science, 73(4), 600-604.

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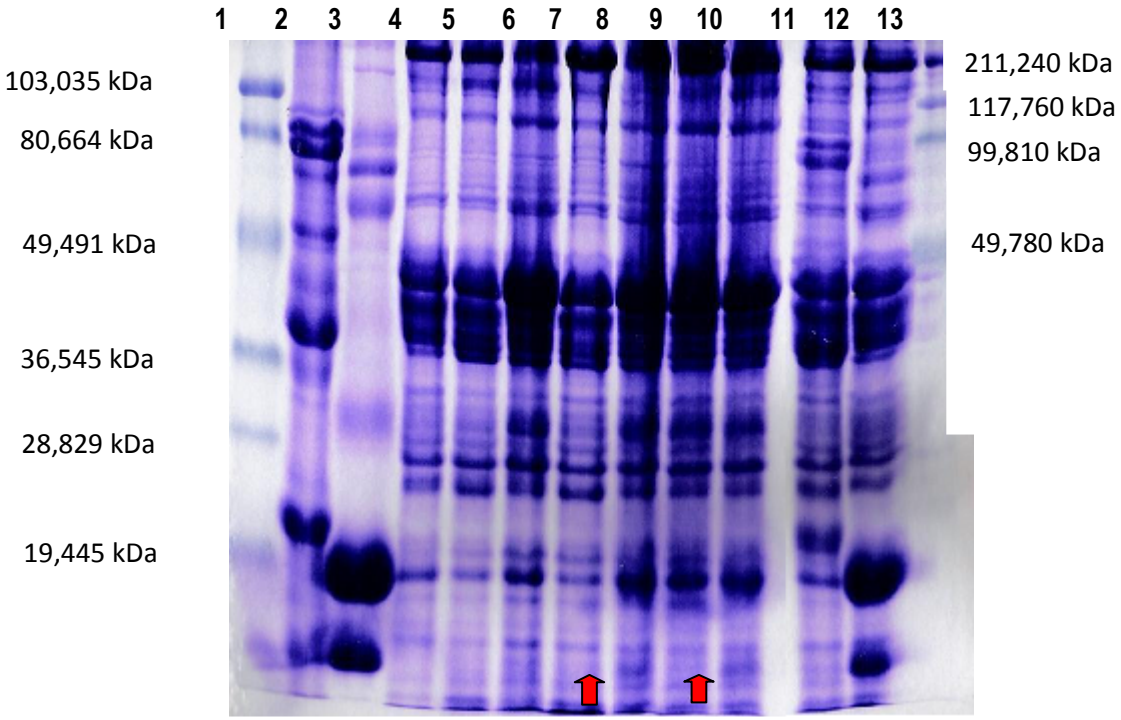
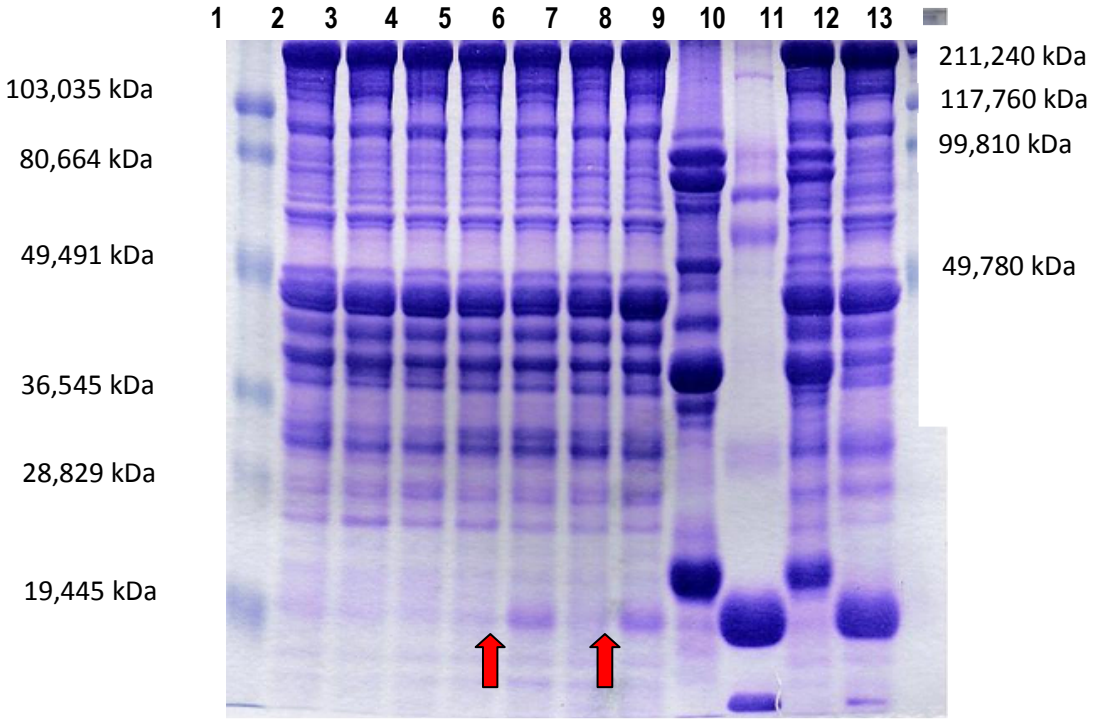
Figure 1. SDS-PAGE Electrophoretic pattern of pork loin enhanced with non-meat proteins and sodium tripolyphosphate. Extraction with Urea 6 M. Narrows point to WPC protein band markers that appeared in enhanced meat.



Lanes 1 and 13: molecular weight standards; lane 2: SPI; lane 3: WPC; lanes 4 to 10: experimental treatments; lanes 11 and 12: positive controls.

Figure 2 – SDS-PAGE Electrophoretic pattern of pork loin enhanced with non-meat proteins and sodium tripolyphosphate. Extraction with Tris-HCl-SDS-ME. Narrows point to WPC protein band markers in that appeared in enhanced meat.



Lanes 1 and 13: molecular weight standards; lanes 2 to 8: experimental treatments; lane 9: SPI; lane 10: WPC; lanes 11 and 12: positive controls.