Point-of-care extraction and GELFrEE electrophoresis for pork protein fractionation

Rituparna B.[1], Naveena B.M.[1], Subhasish B.[2], Sowmya D.[1], Barbuddhe S.B.[1], Patra G.[2], Bhattacharyya D.[2]

[1] National Research Centre on Meat, Chengicherla, Hyderabad, India
[2] West Bengal University of Animal and Fishery Sciences, Kolkata, India

Introduction: Common procedure for meat protein extraction involves denaturing solutions containing urea, thiourea, reducing agents, detergents, and salts [1] followed by tissue homogenization and centrifugation under refrigerated condition. Gel eluted liquid fraction entrapment electrophoresis (GELFrEE), a molecular weight-based separation system allows proteins to be constantly eluted from the gel column followed by their collection in the solution phase [2, 3]. This work was conceptualized to optimize a simple protein extraction method by trituration and filtration at field condition and size-based solution-phase GELFrEE fractionation of total proteins from pork under raw and cooked conditions followed by MS analysis to identify few important proteins.

Materials and methods: Authentic pork samples were obtained from retail shops and half of the meat samples were sealed in LDPE bags and cooked in boiling water bath at 100 °C for 30 min. Extraction of proteins were optimized with four extraction buffers; buffer A (urea, thiourea, CHAPS, DTT, carrier ampholytes and protease inhibitor), buffer B (phosphate buffered saline), buffer C (phosphate buffered saline with SDS) and buffer D (phosphate buffered saline with EDTA) under field condition by maceration and filtration method. For GELFrEE fractionation, protocol provided by Expedeon Ltd. was followed with 5% cartridge for protein separation into 12 fractions. The GELFrEE fractions was subjected to SDS-PAGE with midi-electrophoresis apparatus using 12% gel [4]. In-gel trypsin digestion was carried out as per the procedure of Shevchenko et al. (2006) with several modifications followed by analysis in linear, positive ion mode on the MALDI-TOF ULTRAFLEX III.

Results: All the buffers used in the current study were efficient in extracting the proteins from pork, however, based upon the extraction capacity in both raw and cooked condition, the buffer containing PBS and SDS was chosen as an optimum composition suitable for field level extraction using simple trituration and filtration. The protein extract was further fractionated by novel GELFrEE electrophoresis using 5% proprietary cartridge with a fractionation range of 3.5 to 500 kDa. Small molecular proteins are eluted first from the gel and subsequently confined in the collection chamber. Few GELFrEE fractions were further characterized through MALDI-TOF MS, which led to identification of the proteins namely, Thiosulfate sulfurtransferase isoform X2 and Centromere protein X isoform X6 with a corresponding MW of 33647 and 8902 daltons, respectively. These are low-MW proteins eluted as a single band through GELFrEE fractionation and have the potential to be identified as species-specific biomarkers that will help in addressing authentication of meat and meat products. These findings indicate that targeted purification of single protein from a complex meat matrix may be achieved through simple GELFrEE fractionation [5].

Conclusion: In the present study, we optimized the point-of-care protein extraction protocols suitable for isolating pork proteins for further identification and characterization studies. The study has also developed a novel GELFrEE method for simple, rapid and in-solution fractionation of raw and cooked pork proteins. The developed protocols may be easily integrated for different proteomic studies.

Funding: This work was supported by Department of Biotechnology (DBT), Government of India (BT/PR27198/PFN/20/1333/2017).

References:

[1] Della Malva, A., Albenzio, M., Santillo, A., Russo, D., Figliola, L. & Caroprese, M. (2018). Methods for Extraction of Muscle Proteins from Meat and Fish Using Denaturing and Nondenaturing Solutions. Journal of Food Quality, 2018, 1–9.

[2] Tran, J.C. & Doucette, A. A. (2009). Multiplexed size separation of intact proteins in solution phase for mass spectrometry, Analytical Chemistry, 81, 6201-6209.

[3] Banerjee, R., Naveena, B. M., Dasoju, S. & Ande, S. (2021). OFFGEL and GELFrEE fractionation: Novel liquid-phase protein recovery strategies in proteomics studies. TrAC Trends in Analytical Chemistry. 140, 116282.

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

[5] Naveena, B. M., Banerjee, R., Muthupalani, M., Barbuddhe, S., Dasoju, S. & Varakumar, P. (2020). Optimization of novel GELFrEE fractionation for molecular weight-based in-solution protein separation from buffalo meat, pork, and chicken. Food Analytical Methods. 14, 88-97.