P - 133

Application of Proteome Analysis in Meat Science

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The activity of individual genes may be up or down regulated, or switched on or off altogether. These continual adaptations that result in the animal phenotype are ultimately a reflection of the way each animal has expressed their individual genes up until that point in time, and results of consequent protein turnover(that includes proteolysis pathway). Proteomics refers to the whole-scale study of proteins within the cells that make up animals. This area is a rapidly developing field of science that involves identifying and monitoring the expression of individual proteins on a cell-wide level, as well as determining how these proteins interact with each other. Understanding the biology behind the expression of traits and pre- and post-harvest proteolysis enable us to determine the reasons why we see such diversity within herds which are ultimately linked to meat quality even upon during chiller ageing period. The current technology allows us to observe changes in the pattern of protein expression within animals and changes postmortem period. With putting an ultimate goal on assessment and prediction of meat quality, the current experiment was designed to optimize proteome analysis technique for skeletal muscle. Given to the fact of complexity and nature of skeletal muscle where ca. 70% of proteins are composed of contractile proteins (eg. myosin and actin), there is an unfortunate limitation in separation of skeletal muscle proteome by applying 2DE technique. To solve this problem, we attempted to segregate cellular components into cytosol, mitochondria, nucleus, cell membranes, but we encountered a shortage of repeatability. Another simple attempt was made for a separate analysis for water soluble and non-soluble fractions, but this effort did not result in any remarkable improvement. Bearing in mind that sample preparation for proteme analysis is the most critical process and that should be as simple and fast as possible, we returned to analysis whole extract in spite of the limitation in capacity of sample loading. For analytical separation with active loading, a total of 120 ug whole extract appeared to be appropriate for 24 cm × 20 cm system, followed by silver nitrate staining. For preparative gels with CBB G250 staining, 400 ug seems to be acceptable. Upon this condition, *pI* 6.6-8.5/35-50 kDa region was not clearly dissociated whereby a large number of kinase chains and dehydrogenases are populated. However, these amounts appeared to be necessary for other minor copy of proteins and downstream MS work. Addition of DNase and RNase did not improve separation property. Either 60 or 80 kVhrs for from 100 to 600 ug proteins showed a similar focusing pattern. When these conditions were applied to pig longissimus muscle, ca. 1000-1500 spots were detected. In terms of number of spots and intensity during 7 d chiller ageing period, there were great changes in overall profile. When that was compared with 2DE database on the ExPASY that included many kinases, contractile apparatures and cytosol proteins. For more detailed profiles, peptide mass fingerprint works are under progress.