Site of Evaluation. Where do we evaluate pork lean? The loin is difficult because it is not ribbed in practice. The current NPPC standards are for the 10th rib and we seldom see this location! We can't evaluate at the shoulder loin separation (3rd rib) as it is often paler PSE-looking while the rest of the loin is normal. Discussion stated that we can apply the established color to any point (any lean) within the carcass. Just establish a standard description for pork lean and apply it wherever needed.

What About Using ‘A’ and ‘B’ Values?
• Could identify an “off” color.
• Early studies say that L value is the best. The a and b values have been poor historically.
• a and b have an angle, chroma/saturation index. i.e. common L value will have a different ‘a’ and a common ‘a’ will probably have different L values.
• Not sure how do ‘a’ and ‘b’ values change down the line i.e. at the retail case.

The unanswered questions: What is the objective standard? What reference coordinates? What is the purpose of a color standard for pork? How many colors are necessary? Color standards that are too close together to be distinguished with the eye indicates there are too many. Also, if you have too few, the categories are too far apart. How do we deal with variation within the sample (i.e. within the loineye)?

Reference

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RMC RECIPROCATION SESSION

Methods to Monitor/Quantify Postmortem Changes in Muscle Proteins

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Topic Summary
Postmortem changes that occur in muscle are extremely complex. As the cellular and subcellular environment is altered, muscle proteins undergo very dramatic changes. In order to develop an understanding of the specific changes occurring in postmortem muscle proteins many questions must be asked. In order to answer these questions, numerous different biochemical tools can be used. The choice of which biochemical tools to use depends on the parameter to be measured. Some of the most often monitored postmortem changes include protein denaturation and protein degradation.

Denaturation of protein is defined as any modification of conformation not accompanied by the rupture of peptide bonds involved in primary structure. This can have several consequences including the unmasking of hydrophobic groups which can lead to decreased protein solubility, precipitation of proteins onto myofibrils, loss of the protein’s biological activity, and alteration of the susceptibility of the protein to proteolysis. One method to measure protein denaturation on a relative basis involves monitoring the solubility of muscle protein fractions in buffers of defined ionic strengths. After dispersal of the protein in buffer and centrifugation to pellet insoluble cellular fractions, the protein content of the supernatant is determined. The lower the protein content of the supernatant, the less soluble protein is found in that fraction and the assumption is made that the protein in that fraction is relatively more denatured when compared to samples with a higher protein content in the supernatant.

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Another postmortem change in protein that is often monitored is protein degradation. Protein degradation can be defined as the disruption of peptide bonds that results in protein fragments that may or may not retain biological activity. Many different methods can be utilized to monitor the degradation of myofibrillar protein. A rapid, inexpensive, and relatively simple method is the Myofibrillar Fragmentation Index (MFI). The basis of this method is to measure the turbidity of a suspension of myofibrils in solution. As myofibrillar protein is degraded, myofibrils fragment, which results in greater turbidity of the solution in which they are suspended. This method can give an overall gross estimation of the relative amount of proteolysis that has occurred in a sample. However, one cannot gain an appreciation of which particular proteins are affected.

In order to achieve an understanding of which specific proteins are altered by postmortem degradation, more sophisticated methods must be employed. One method that can provide investigators with more detailed information on which proteins are affected by degradation is Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Electrophoresis depends on the fact that charged molecules move in response to an electrical field. This movement is affected by net charge and by resistance. SDS-PAGE utilizes sodium dodecyl (lauryl) sulfate (SDS) not only to solubilize protein, but also because it binds to protein and provides a large net negative charge. This negates the effect of native charges on proteins in solution. Therefore, movement of proteins in SDS-PAGE systems is primarily due to size, with larger proteins migrating near the top of a SDS-PAGE gel and smaller proteins migrating progressively further down the gel. The extent of migration, the amount of separation of individual protein bands, and the molecular weight range of the proteins examined is altered by the pore size that is chosen. Polyacrylamide, the matrix that is used in SDS-PAGE systems, is ideal for this application as it is easily reproducible, chemically inert, stable over a wide range of pH, temperature and ionic strengths, transparent, and can be easily manipulated to obtain a wide variety of pore sizes. The pore size can be altered in two main ways; changing the percentage of total acrylamide in the gel solution, and altering the ratio of acrylamide to its cross-linker, bisacrylamide. Changing the percentage of acrylamide (usually within the range of 4 to 20% acrylamide) can have a dramatic effect on the molecular weight range of proteins that are resolved. Changing the ratio of acrylamide to bisacrylamide (usually within the ranges of 37.5:1 to 200:1, acrylamide:bisacrylamide) will generally have a more subtle effect and might be utilized when only slight modifications in the amount of separation between protein bands is desired.

Visualization of the separated proteins within a SDS-PAGE gel is achieved by using a staining system that either stains the protein bands themselves, or stains the gel matrix surrounding the protein bands (negative stain). Two of the most commonly used direct staining methods are Coomassie blue R-250 and silver staining techniques. Coomassie is usually the method of choice when a simple, consistent method is desired and the protein band of interest is present in at least 30-50 ng quantities. If more sensitive method is desired, silver staining is often the method of choice. Both of these methods are fixative in nature and are generally considered to be terminal steps. If further processing of the protein bands is desired, alternatives include negative staining methods (i.e. copper and zinc staining methods). These methods are rapid (usually staining is completed in approximately ten minutes), sensitive (often 6-10 ng can be detected), and allow the researcher to continue on with other procedures such as immunoblotting.

SDS-PAGE is a tool that can provide substantial qualitative information about a sample. Caution should be taken when attempting to quantify SDS-PAGE gels. It must be noted that quantification of protein bands on SDS-PAGE gels (usually by densitometry) must involve the use of internal standards. Since different proteins can bind different amounts of dye, the best internal standard to use is a known quantity of a purified sample of the protein of interest. Quantification of a particular protein band is then relative to this internal standard.

SDS-PAGE does have limitations. Identification of a particular protein band is limited to comparing its migration relative to the migration of specific molecular weight standards or to purified proteins. This limitation can make it difficult to identify the origin of specific polypeptides arising from the degradation of proteins. A powerful extension of SDS-PAGE that can often overcome some of these limitations is immunoblotting (Western blotting). Immunoblotting depends upon the immunodetection of specific proteins that have been electroblotted to a membrane. Immunoblotting begins with the separation of proteins using PAGE techniques and continues with transfer of the proteins to a membrane (usually nitrocellulose or PVDF[polyvinylidene difluoride]). After the remaining protein binding sites on the membrane are blocked with a solution of irrelevant, non-reactive protein (usually BSA, non-fat dry milk or gelatin) to prevent non-specific binding of subsequently applied antibodies, the membrane is exposed to a solution containing an antibody specific for the protein of interest. This antibody (primary antibody) binds to its target protein and it is the presence of this antibody that is detected with subsequent detection steps. Occasionally the primary antibody is directly conjugated with a reporter molecule that can be activated to produce either a colorometric signal or an emission of light that can be captured on autoradiography film or by phosphorimaging devices. More often, detection of the primary antibody is accomplished by application of a secondary antibody that is specific for the primary antibody. It is this secondary antibody that is tagged with a reporter molecule that can then be activated.

Immunoblotting has advantages over SDS-PAGE as proteins can be identified more accurately with antibodies specific for a particular protein than they can be by depending solely on the relative migration of a protein band. In addi-
It is often possible to identify the origin of a protein fragment by its immunoreactivity.

Immunoblotting also has limitations. In most cases it is best used as a qualitative tool. Just as with SDS-PAGE, an internal standard of the protein of interest must be included on every blot if quantitation is desired in order to account for any variation in transfer efficiency, antibody recognition, etc. In summary, there are a wide variety of techniques that can be used to monitor changes in muscle proteins. The choice of a particular method depends greatly upon the question to be answered and the advantages and/or limitations of the methods available.

Discussion Summary

Compare the time to use the original method of blotting technique and absorption versus electrophoretic techniques. With myofibrillar proteins, passive transfer will not result in the desired efficiency of eluting proteins on the gel. The tank method of immersing gels will give superior results. You cannot get sufficient transfer by only flowing fluids through it.

What are the advantages and disadvantages of chemiluminescence? It takes of time to switch systems to chemiluminescence. One should be careful with controls and standards used. It is difficult to use imaging systems to capture reproducible results using chemiluminescence. Better results can be achieved with I-125. Making the switch from color to chemiluminescence or, from film to phosphorimaging requires much experimentation.

There was good discussion on elucidating titin. It was suggested that the transfer of high molecular weight proteins should include mercaptoethanol to elute large proteins like titin. Titin is difficult to transfer completely out of a gel and will require 5 to 6 hours in a cold room, consistent buffer, and uniform time and voltage. There may be difficulties with fragments running through the membrane and PVDF may help to retain small fragments. It is difficult to have a standard for large proteins like titin and for all isoforms of a protein such as troponin-T.

What could be the application of these techniques to cooked/processed meats? It is difficult to solubilize heated myofibrillar proteins. Most myofibrils dissolve in SDS or urea/thiourea. You would need to destroy product physically as much as possible and not just rely on dissolution.

When should a stacking gel be utilized? With 5%, you generally don’t use a stacking gel; anything greater than 7% does require a stacking gel. It should be two times higher than the amount of the sample.

What is the availability of antibodies for muscle tissue? There is a great bank of monoclonal antibodies available and some researchers will share theirs. It can sometimes take a significant quantity of antibody.

There was a short discussion on more automated or simpler procedures that are under development. There have already been technique and material improvements.

Selected References

Protein Solubility


Myofibrillar Fragmentation Index

SDS-PAGE and Immunoblotting


