

IMPROVEMENT OF PORK MEAT QUALITY WITH PROTEOMICS



Is the pork meat you eat tender, flavorful and juicy? Have you ever asked yourself how much is the nutrition value of it?
At the moment there is no way to ensure whether the meat pieces that the consumers are buying are classified as 'QUALITY'.
So wouldn't it be helpful to have a tool that could check the quality of the meat using different biomarkers as indicators?

Introduction and Aims

Pork is the predominant meat consumed in the world. Industries of pork sector now include the quality traits of meat as an integral part of selection programs, as it has become the most important factor in this increasingly competitive market. Identification of biomarkers whose expression or abundance is associated to a phenotypic trait of interest could be used to predict such sought traits. However, meat quality has to be optimally aligned with the lowest cost in production. As a result, expected outcomes are to provide control tools of meat quality evaluation usable in livestock sector and meat industry.

The main **objectives** of this review are:

- To find the association of the most common identified proteins in the latest proteomic studies in the pork muscle with a quality trait.
- Evaluate which ones could be used as indicators for the selection programs.
- Then, from the collected data, design a process in purpose for the application within the meat industries. The tool purposed here is an Ab Chip which could be used for a routine evaluation.

What is Meat Quality ?

Pork quality traits result from interactions among:



Pork eat quality covers technological and sensory dimensions, including:

Drip loss	Color
Intramuscular fat (IMF) content	Post mortem pH
Tenderness	Juiciness
Flavor	Wholesomeness
Visual appearance	Nutritional value
Texture	Lean



Proteomics for the search of Biomarkers of Quality

The proteome is dynamic and varies with the physiological state of the organism making proteins suitable to represent the final result of a complex gene expression system while considering the post transcriptional modifications. As a result, biomarkers of gene expression appear more accurate than genetic markers to predict the meat quality variations.

Workflow analysis

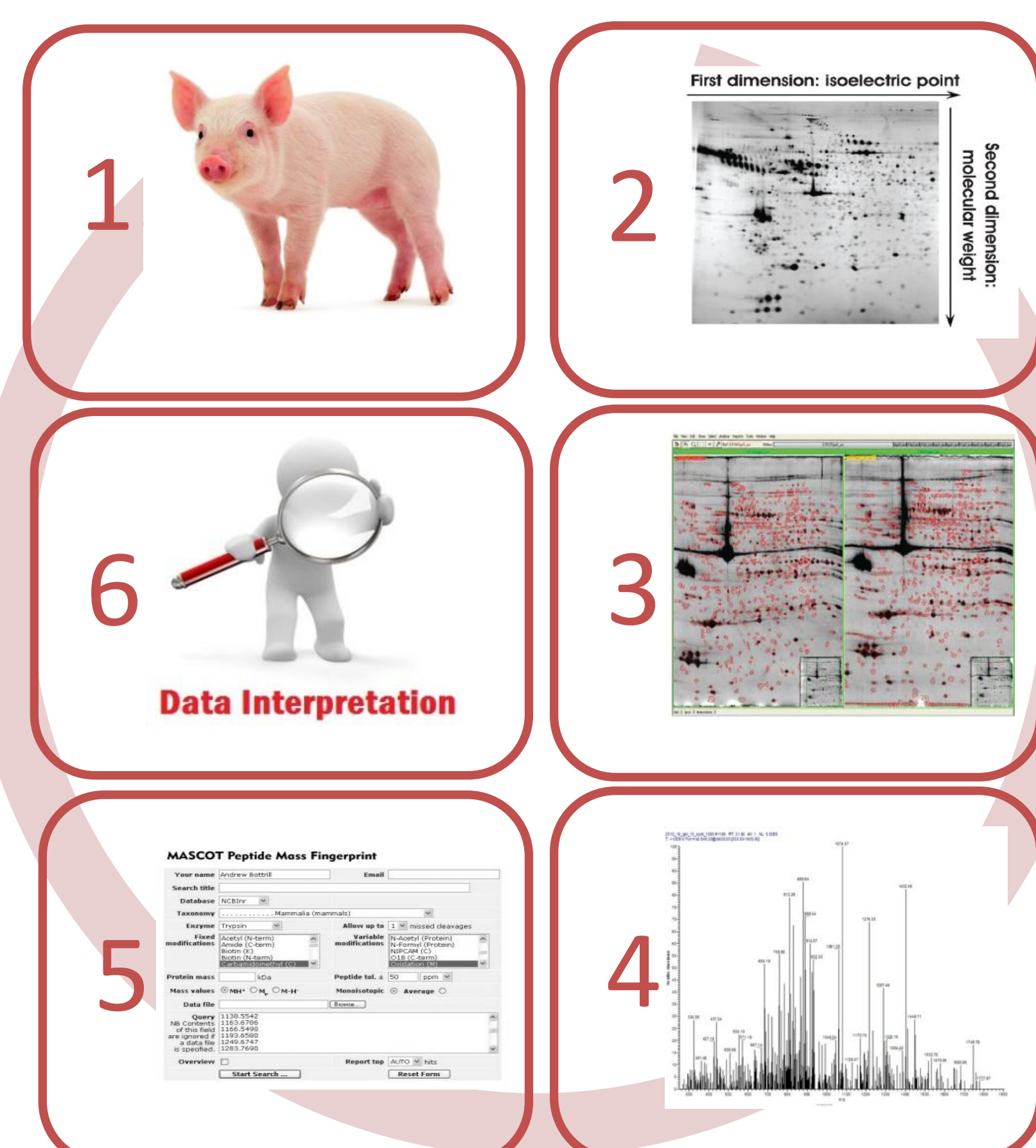


Fig. 1. Schematic drawing of the different steps in a proteome analysis using 2-DE and mass spectrometry.

- Animals chosen for the analysis. The analysis in meat proteomics are performed on muscles from different pigs (high quality and low quality) or reared in different conditions so that a broad range of meat qualities and variety of gene expression profiles can be obtained.
- Protein extraction and 2-Dimension Electrophoresis.
- Image and statistical analysis.
- Evaluation of the data and selection of significantly changed proteins.
- Extraction of the changed spots and identification of the proteins by MS spectrometry.
- Interpretation of the results which often leads to new hypothesis and new cycles to be performed.

A case Study: Ractopamine administration influences muscle proteomic profile

Dietary ractopamine (a β -adrenergic agonist feed additive) improves pork leanness. This particular study examined the influence of ractopamine on the sarcoplasmic proteome of post mortem pork Longissimus thoracis muscle. Proteome profile was analyzed using 2D eletrophoresis and mass spectrometry from 2 groups: CON (control) and RAC (whose diet included ractopamine). Nine protein spots were found to be differently expressed in abundance between the 2 groups.

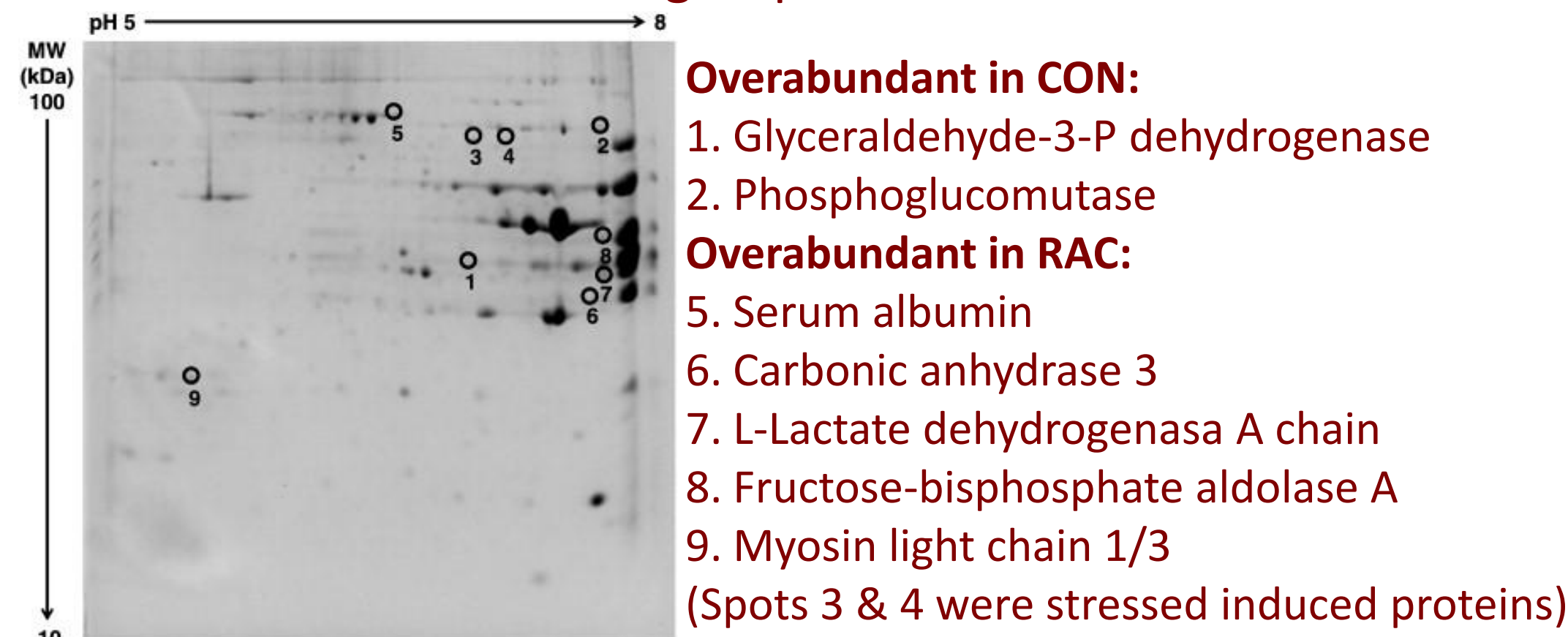


Fig 2. Coomassie-stained two-dimensional gel of the sarcoplasmic proteome extracted from pork *Longissimus thoracis* muscle. Nine protein spots differentially abundant in control and ractopamine-fed pigs are encircled.

- Overabundant in CON:**
- Glyceraldehyde-3-P dehydrogenase
 - Phosphoglucumutase
- Overabundant in RAC:**
- Serum albumin
 - Carbonic anhydrase 3
 - L-Lactate dehydrogenasa A chain
 - Fructose-bisphosphate aldolase A
 - Myosin light chain 1/3 (Spots 3 & 4 were stressed induced proteins)

A total of four proteins out of the ones overabundant in RAC were correlated with meat quality traits and could possibly be used as biomarkers:

- Serum albuminum:** Antioxidant and binding protein related to color and tenderness.
- Carbonic anhydrase-3:** Protein with low hydratase activity which has been positively correlated with drip loss and color.
- L-Lactate dehydrogenasa A chain:** glycolitic enzyme catalizing conversion lactate to pyruvate. It has been related to dark color meat.
- Myosin light chain 1/3:** Participates in muscle contraction that influences tenderness, intramuscular fat and color.

Collection of Results

Quality traits	Species/muscle	Proteins involved in quality changes
Texture	Pig (LM; Longissimus Muscle)	Actin fragments, MHC fragments, MLC II glycolytic enzyme triose phosphate isomerase I
Color	Pig (LM)	Coflin 2, tropoin T, α -8 crystalline, HSP27 kDa, group chain A aldehyde dehydrogenase, glycerol-3 phosphate, dehydrogenase, haemoglobin α -chain, β 1 globulin
WHC (drip loss)	Pig SM (Semimembranosus)	ATP synthase δ subunit, NADH dehydrogenase, succinate dehydrogenase, enolase 1, enolase 3, glycerol-3-phosphate dehydrogenase, haemoglobin α and δ -chain, HSP27, RB-crystallin, glucose-regulated protein 58 kDa, low-molecular-weight protein tyrosine phosphatase, S-transferase α , cyclophilin D
Texture	Pig (LM)	Adipocyte fatty acid-binding protein, acyl CoA-binding protein, enoyl CoA hydratase, aldose reductase, triosephosphate isomerase, initiation factor eIF-2B, chaperone subunit 2, profilin II
WHC (drip loss)	Pig (LM)	Creatine phosphokinase M-type, desmin, SWI/SNF-related matrix-associated actin-dependent regulator of chromatin a1 isoform b
IMF	Pig (Intramuscular Adipocytes)	Cytosolic malate dehydrogenase, isocitrate dehydrogenase, enololases, aldolases, perlipin, long-chain fatty-acyl CoA
Color	Pig (L. lumborum)	Myoglobin isoform, HSP 72, CK, calcium-binding protein, cytosolic glycerol-3-phosphate dehydrogenase, dimeric dihydropyridyl dehydrogenase, isoform M1 of pyruvate kinase
WHC (drip loss)	Pig (LM)	A-8 crystalline, HSP 27, HSP 70, HSP 90

Table 1. Proteins involved in the variability of meat quality traits. First section of the table has been modified and extended of Hamill et al. (2012a).

Principal of mechanism of the Ab Chip

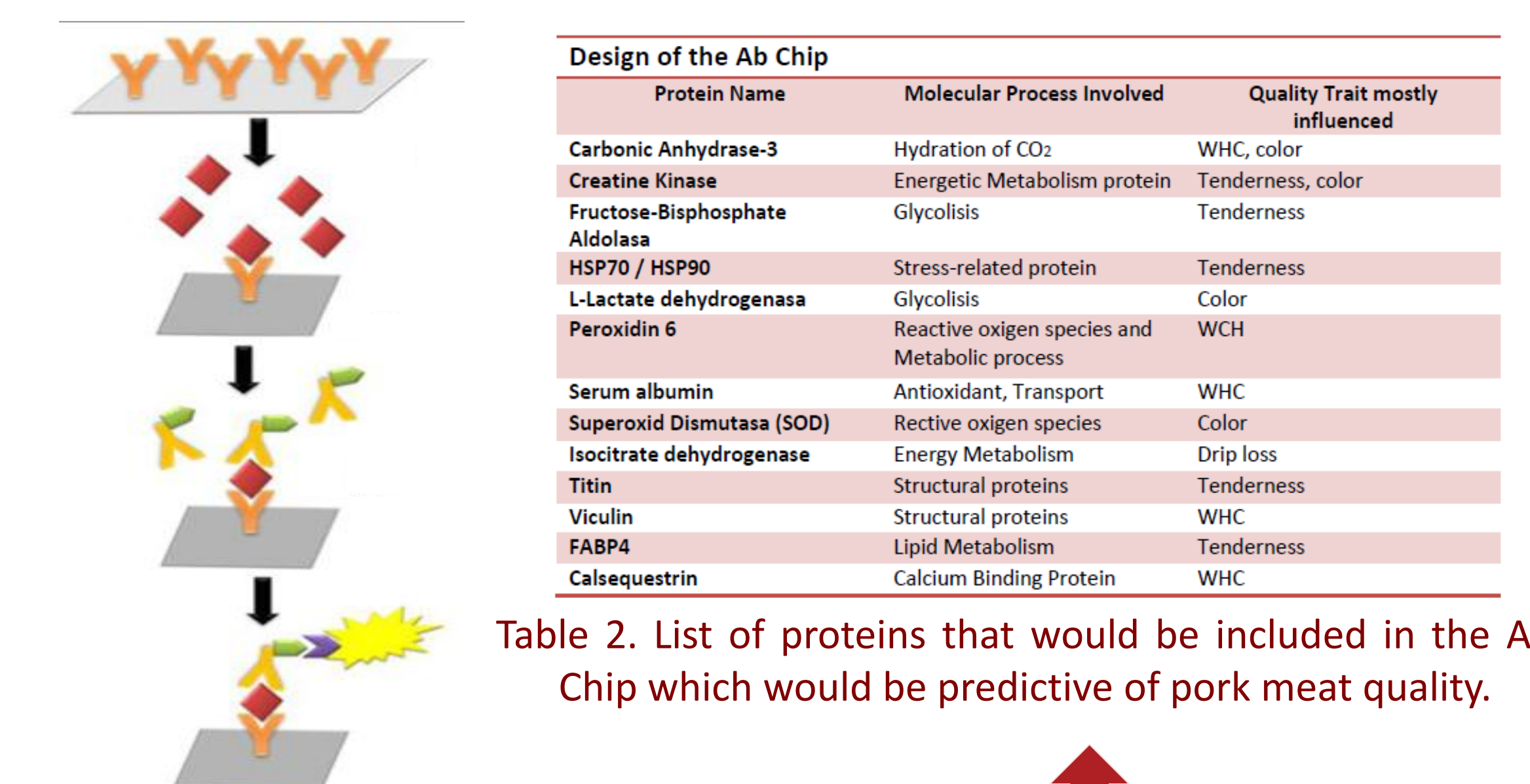


Table 2. List of proteins that would be included in the Ab Chip which would be predictive of pork meat quality.

The Ab chip would give a precise, specific and quatitive measure.

Discussion and Conclusions

- Proteomics is a powerful tool to investigate postmortem protein degradation in meat. Collected results revealed the biological mechanisms involved in the determination of meat quality and provided elements (markers) for these complex traits.
- However, the identified proteins were shared between the traits, meaning that such traits are affected by the expression levels of the same proteins. An accurate certification of the quality would be predictive only with a specific combination of proteomic biomarkers.
- To predict this combination and improve the prognostic value of biomarkers other improved approaches should be used. One possibility would be the integration of proteomics, transcriptomics, and metabolomics, allowing a deeper understanding of the meats fundamental make up.

"We are ready to show the industry what we have to offer. The next step will be to translate the biomarkers to tools and to test them at the slaughterhouses," said Niels Oksbjerg, a well-know scientist of the sector, who hopes to be able to obtain funding for this development work.

My Suggestion of a DESIGN OF AN EFFECTIVE TOOL : The Ab Chip

- This tool could give the competitiveness of the industry by allowing it to provide the consumers with controlled meat quality.

References

- Costa-Lima B., Suman S., Li S., Beach C., Silva T., Silveira E., Bohrer B. and Boler D. (2015). Dietary ractopamine influences sarcoplasmic proteome profile of pork Longissimus thoracis. Meat Science 103, p7-12.
- Gobert M., Sayd T., Gatellie P. and Santé-Lhoutellier V. (2014). Application to proteomics to understand and modify meat quality. Meat Science 98, p539-543.
- Hamill R., Marcos B., Rai D. and Mullen A. (2012a). Omics approaches to meat quality management. Omics Technologies: Tools for Food Science p.249-281.
- Hollung K., Veiseth E., Jia X., Faergestad E.M. and Hildrum K.J.. (2007). Application of proteomics to understand the molecular mechanisms behind meat quality. Meat Sci. 77, p97-104.
- Te Pas M.F.W., Hoekman A.J.W. and Smits M.A. (2011). Biomarkers as management tools for industries in the pork production chain. J. Chain Network Sci11, p155-166.
- The Meat Site. (2012). Scientists Find Meat Quality Biomarkers. Available: <http://www.themeatsite.com/meatnews/16471/scientists-find-meat-quality-biomarkers/>. Last accessed 26 April 2015.