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Integrated Omics Approaches in Meat Science Research

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ABSTRACT

Meat is a nutrient-dense food, and its quality depends on various factors that range from pre- to post-harvest. Any alteration in the conversion of muscle to meat can impact product quality and consumer acceptance. Hence, elucidating the factors that can influence meat quality such as tenderness, water holding capacity, flavor, and color are essential to understand quality defects, limit meat wastage, and ensure consumer satisfaction. The aspects involved in meat quality changes are complex and interrelated. Hence, traditional wet-laboratory techniques may not be able to provide global changes in biomolecular interactions. In recent years, a systems biology approach utilizing genomics, proteomics, transcriptomics, and metabolomics has been incorporated into basic meat science research to understand the mechanistic basis of meat quality and the potential development of biomarkers. The overall goal of this review is to give an outline of various omics techniques and their application in meat science research.

Keywords: *Genomics, Meat quality, Metabolomics, Omics, Proteomics*

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INTRODUCTION

With the growing demand for high-value animal proteins, there is increased pressure on the livestock industry to produce more meat. Although socio-demographic and emphasis on environmental aspects has increased in recent years, limiting food waste remains a top priority to feed the growing population. Meat is a nutrient-dense food, and it is estimated in the United States, Canada, Australia, and New Zealand, approximately 22% of total meat and poultry produced is discarded annually (Gunders, 2012). Meat is a highly perishable commodity; therefore, understanding the biochemical basis of quality changes is critical to limit meat wastage without compromising consumer expectation on quality (Ramanathan *et al*., 2020). Meat is biochemically active (Fig. 1), and its properties depend on the cascade of reactions that occur before and after animal harvest. Protein, metabolites, DNA, and fatty acids expression can influence meat quality, and its functions are interrelated. Therefore, traditional laboratory techniques may not provide insights into global changes in biomolecules.

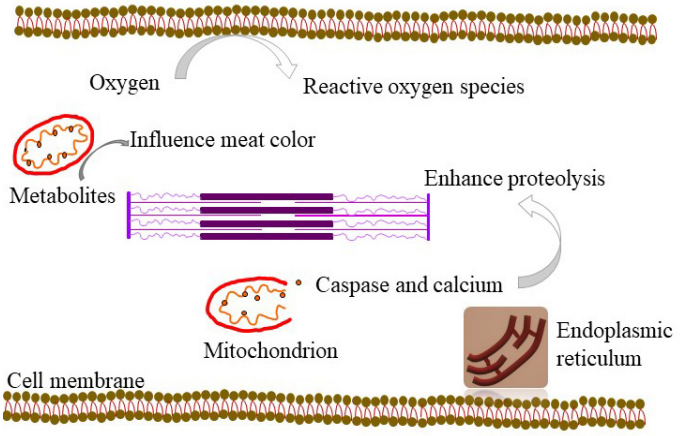

Fig. 1 Schematic representation of different biochemical processes and its impact on meat quality

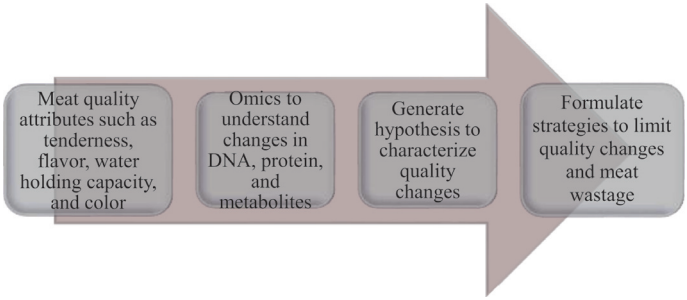

Fig. 2 Usefulness of omics technology in meat quality studies

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Omics techniques are interdisciplinary and apply concepts in various fields such as biology, computer science, and engineering. Although omics techniques will not provide a definite answer to a question, a discovery-driven approach helps to develop hypotheses to a complex problem (Fig. 2). This review aims to discuss important meat quality attributes and how omics technology can be used to understand meat quality changes.

FACTORS AFFECTING MEAT QUALITY

Immediately after slaughter, the metabolism changes from aerobic to anaerobic (Ouali *et al*., 2013). The approximate postmortem muscle pH of depending on species ranges from 5.6 to 5.8. Any alteration in pH drop can influence meat quality (English *et al*., 2016). More specifically, the amount of glycogen in muscles prior to slaughter and post-harvest factors such as storage temperature can influence the activity of enzymes and associated formation of metabolites. pH is an inherent biochemical factor that can affect water holding capacity, tenderness, and color (Hughes *et al*., 2019).

IMPORTANT MEAT QUALITY ATTRIBUTES

Tenderness and flavor: Tenderness has been valued as the most important factor influencing meat quality, as has been deemed critical for consumer repurchase decisions (Bailey,1972; Maltin, 2003). One of the essential factors that influence tenderness is the age of animals. In several studies, consumers are willing to pay more for steaks that are guaranteed tender (Lusk *et al*., 2001; Miller *et al*., 2001; Feuz *et al*., 2004). Lusk *et al*., (2001) reported that consumers were willing to pay an average of $2.71/kg more to purchase a "tender" rather than a "tough" steak. Additionally, Miller *et al*., (2001) estimated that consumers were willing to pay a premium ranging from $0.59/kg to $1.23/kg for steaks in the tender category as opposed to the intermediate to tough categories. Feuz *et al*., (2004) reported that with every kg increase in WBSF, a consumer's willingness to pay decreased by $0.24/pound. This follows the trend of previously mentioned studies that concluded as tenderness decreased, a consumer's willingness to pay also decreased. Moreover, Lusk *et al*., (2001) determined that sharing information about the predicted tenderness of a product (i.e., on the label) significantly impacts the consumer's preferences and willingness-to-pay. Consumers are willing to pay more for a product that can be guaranteed tender. This is a step in the right direction for producers to have an incentive to produce products that can be guaranteed tender. The United States Department of Agriculture (USDA) has a verified program "USDA Tender" where a commercial facility can randomly test lots of beef and if the sample selected is 4.4 kg or less when tested by WBSF it can be marketed with the official USDA Tender shield (ASTM F2925-11). With more research and producers willing to pay for the WBSF testing this program can add a vital incentive for ranchers to produce more tender beef.

There are several ways to predict tenderness. As mentioned previously, WBSF can be used to determine the kilograms of force required to shear through a steak. However, this process required the steaks to be sacrificed from the supply chain to be tested, and the process is more than 24 h from cook time to shearing. Another method that is commonly used it Slice Shear Force (SSF). The WBSF and SSF, while viable options in a research setting, are not practical for industry. Instead, vast research is being conducted to discover a non-invasive method of sorting carcasses in commercial facilities. The most common methods being research are imaging technologies, since cameras are currently used in grading U.S. beef, the most obvious means of adding another tool would be to the already existing cameras. More specifically, Naganathan *et al*., (2008) concluded that near-infrared hyperspectral imaging could be used to predict beef tenderness accurately. Hyperspectral imaging uses more wavelengths than the traditional red, blue, green to analyze each pixel of the image. By analyzing these pixels, the research can predict what is present and how much of it is present. This process has been proven at a 96.4% accuracy in a research setting for sorting steaks into three tenderness categories; tender, intermediate, and tough (Naganathan *et al*. 2008). Further studies were conducted to determine near-infrared hyperspectral imaging as a reliable source to sort carcasses based on tenderness (Elmasry *et al*., 2012).

Some important things to consider when measuring tenderness are the extrinsic and intrinsic factors influencing tenderness. Intrinsic factors would include the age, sex, breed, and genetics of the animal, while the extrinsic factors would include the pre mortem stress/handling, the slaughter process, and postmortem handling. While it is hard to control the intrinsic factors on an entire lot of cattle, it is easier to limit the variability in the extrinsic factors. Many studies have been conducted to prove the current techniques of the industry are the most effective. For example, the chilling process of carcasses has been improved to limit the occurrence of cold shortening, which is a major contributor to tough beef. The intrinsic factors are more commonly evaluated when tenderness measurements are taken.

The amount of collagen in a product has a significant impact on the shear force values. As animal ages, the amount of collagen cross-linking increases (Bailey, 1989). The sex of the animal also has some implications in the amount of collagen

cross-linking, and its effect on tenderness. Castrated males typically have a higher collagen turn-over rate, so less mature cross-links, and therefore are typically more tender (Bailey, 1989). While the genetics of the animal is still largely unknown, there is some idea about how the breed of an animal affects the collagen driven tenderness. Many of the smaller earlier maturing breeds, apart from Angus, tend to have higher concentrations of mature cross-links upon harvest. While the larger later-maturing breeds, including *Charolais* and *Limousin*, tend to have a lower percentage of mature collagen cross-links and therefore are typically more tender (Christensen *et al.* 2011).

Water holding capacity: The ability of postmortem muscle to hold water with the application of external force is an important quality attribute. Lower water holding capacity can lead to greater drip loss and can affect product quality. Mainly protein components of meat hold water; hence, any changes in protein can influence water holding capacity. Omics can study the relationship with proteins responsible for water holding capacity and also to understand proteolysis.

Water holding capacity can be measured through a variety of methods to determine total moisture, free water, and bound water. Total water content can be determined by drying with a microwave oven or a vacuum oven. These methods include heat to evaporate the water evaluating the weight of the sample before and after drying. Near-infrared (NIR) technology is an AOAC-approved rapid method to determine percent moisture, and this technology is found in the FoodScan FOSS equipment (FoodScan Lab Analyzer, Serial No. 91753206; Foss, NIRsystem Inc.; Slangerupgrade, Denmark). Using the FoodScan, the measurements are rapid and easy to obtain. Near-infrared measures the infrared light reflected from the sample by the molecular vibrations of water. The percent moisture is determined by the difference between the wet and dry sample weights divided by the wet sample weight. Wierbicki and Deatherage (1958) established a method to determine water holding capacity by determining total moisture, free water, and bound water. There has also been a new procedure and instrument for measuring the water holding capacity of fresh meat and includes a new instrument and different parameters as an improvement upon the filter paper press method. Barbera (2019) measured raw meat total moisture content from samples of *longissimus thoracis*. For cooked meat, cooling loss, and residual water was obtained by compression to assist in measuring the hardness (Barbera, 2019).

Unlike fresh meats, processed meat systems can have a variety of different ingredients added to the food matrix, which can greatly affect the water holding capacity. Many processed types of meat products can be injected with brine or placed in a marinade, which can be utilized to improve the water holding capacity of the meat system dependent upon their ingredients. It has been previously seen that electrical conductivity of salt solutions of sodium chloride, sodium bicarbonate, and sodium tripolyphosphate can be utilized as parameters of predicting the WHC of marinated chicken breast meat (Kaewthong and Wattanachant, 2018). According to the American Meat Science Association, starches are no longer just used for water binding and retention purposes; while they do fulfill these roles, the starches can play a large part in consistent with juiciness, tenderness, texture, and improve the yields and hold times, all things which were happening before, but now being studied at a more precise level, which is a true implication of the necessity of a well-balanced water holding capacity level in a meat system (Swenson and Katen, 2004).

Meat color: Meat color plays a role in the purchasing decisions in countries where meat is marketed as fresh (Mancini and Hunt, 2005; Carpenter *et al*. 2001). Depending on the concentration of myoglobin, meat color can range from pink to dark-red. A bright cherry-red color is preferred by consumers, and any deviation from a bright-red color can leads to less consumer acceptance or discounted at the processing plant (Carpenter *et al*. 2001). Dark-cutting beef is an example of a color deviation, which has a worldwide occurrence. In the United States, approximately 1.9% of carcasses are graded as dark-cutting costing the industry $91-251 million based on the 2016 National Beef Quality Audit, while in Canada, according to the 2016/2017 National Beef Quality Audit, dark-cutting carcasses cost the cattle sector $10.6 million (https://www.beefresearch.ca/files/pdf/NBQA-Carcass-Audit-Mar-27-2018-F.pdf). Meat color is primarily due to myoglobin, and depending on the state of myoglobin, it can impart bright cherry red (due to oxymyoglobin), dark-red (due to deoxymyoglobin), or brown (due to metmyoglobin). Meat discoloration is inevitable, but, understanding the factors that can limit discoloration such as maintaining cold chain during storage or transport and limiting oxygen content can extend color life (Faustman *et al*. 2010).

The American Meat Science Association Color Guide has provided detailed instructions about color measurements and precautions (AMSA, 2012). Meat color can be evaluated through instrumental and visual panel approaches. Both benchtop and handheld spectrophotometers can be used for color evaluation. One of the most common ways to reporting color is utilizing the CIE Tristimulus Values (CIE L^* a^* b^*). The CIE L^* values provide for the lightness of the meat product

on a scale of 0-100 (black-white). From the spectral data from 400 to 700 nm can be used to calculate percent deoxy-, oxy-, and metmyoglobin. Meat color is determined by two inherent biochemical characteristics, such as oxygen consumption and metmyoglobin reducing activity (Tang *et al*. 2005; Ramanathan and Mancini, 2018). Oxygen consumption is related to bloom development, and metmyoglobin reducing activity predicts color stability. Oxygen consumption is estimated by the change in deoxy- or oxymyoglobin from pre- to post-anaerobic conditions allowing for an understanding of mitochondrial oxygen consumption of the product. Additionally, metmyoglobin reducing ability can be understood by the decrease in metmyoglobin through reduction pathways over time, contributing to the understanding of color stability.

When evaluating meat color, there are several precautions to consider for instrumental and visual evaluation. The display case or cooler where the product is being held should not be on defrost when evaluating meat color to limit the formation of condensation. The packaging type is an important consideration as well. The packaging film used should also be used to standardize the instrument to limit the effects of the film type and thickness on the reflectance and absorbance. In modified atmosphere packaging, the meat product has to be flipped to evaluate the instrumental meat color; therefore, it is important to limit the accumulation of moisture and fat on the film surface, which may disrupt color. When evaluating whole muscle product, care should be taken to limit the evaluation of high fat or marbling area to better determine the lean muscle color.

IMPORTANCE OF OMICS IN MEAT SCIENCE RESEARCH

The term omics refers to a field of study in biology mainly aimed at collective characterization and quantification of pools of biological molecules that translated into structures, function, and dynamics of organs/organisms. Modern omics techniques mostly utilized in cell biology, biochemistry, and physiology are currently utilized in meat science research to understand the molecular mechanisms underlying meat quality characteristics. Several of these molecular tools more specifically proteomics, metabolomics, and genomics have been employed in elucidating molecular mechanisms that regulate and mediate meat quality traits (Lametsch and Bendixen, 2001; Sayd *et al*. 2006; Joseph *et al*. 2012; Canto *et al*. 2015). The other omics tools such as genomics and metabolomics have also been extensively applied in meat science research (Andersson *et al*. 1998; Straadt *et al* 2014). The basic differences in omics techniques are summarized in Figure 3 and Table 1. Various studies have successfully utilized omics techniques to explain variability or meat quality deviations (Table 2). Further, studies were able to correlate meat quality attributes to omics findings. Hence it is crucial to report sampling details and meat quality analysis in published research.

GENOMICS

Genomics analysis provides information about the intragenomic interactions in the genome. Although the genome of an organism is relatively constant, genomics studies have not revealed a consistent genetic marker with regards to meat quality. Genomics is the study of all genes in an animal or individual. This includes studying the structure and function of genes that affect a quality trait and understand the evolutionary relationship. Gene function determines RNA (transcriptomics), protein (proteomics), and metabolites (metabolomics) formation. The practical application of studying gene may be to develop biomarkers for meat quality traits by sampling animals either before death or postmortem. One of the practical implications is the identification of RYR1 gene mutation (Fujii *et al*. 1991) and associated effects in the development of the pale soft exudative condition.

Each trait is polygenic, hence influenced by various genes. Therefore studying one gene may not provide meaningful information. One of the approaches used in genomics is to identify variations within the DNA sequence among different animals and determine their significance to a meat quality trait. The most common type of genetic variation is known as a single nucleotide polymorphism or SNP. These small differences may help predict a quality variation such as tenderness or pH. The human genome project and associated developments have paved the way to sequence the entire genome in a species. Genome-wide association studies are a relatively new way to identify genes involved in meat quality traits. Each study can look at thousands of SNPs at the same time and helps researchers to pinpoint genes that may contribute to a specific meat quality defects. The genomic analysis includes extraction of DNA from meat, followed by enzymatic digestion to cut DNA into smaller strands. These strands are later incubated with primers to promote binding with complementary bases. With the use of ChiP-sequencing (chromatin immunoprecipitation), several genes can be identified in a single run. This can also generate big data; hence computational software is used to analyze gene sequences. Several genes associated with a quality trait; however, some genes may not be functional. There is an increased interest in quantifying mRNA. Hence, researchers are quantifying mRNA (transcriptomics) to understand quality changes.

Table 1: Summary of different omics approaches and application in Meat Science research

Omics technology	Application in Meat Sci	Platform	Advantages	Limitations
Proteomics (the study of proteins)	To study mechanistic basis for tenderness. Use to determine biomarker tender meat	Gel-based (1D or 2D gel)	Proteins with specific isoelectric points and molecular weights can be targeted.	Low concentration proteins cannot be detected; underrepresentation of extreme acid/basic proteins; labor-intensive
	To study muscle-specific differences in color stability			
	To study differences in water holding capacity	Gel-free	Wider coverage of proteins identified, easier comparison of multiple treatments	Expensive; difficult to identify proteolysis.
	To characterize the binding of lipid oxidation products on protein such as myoglobin			
Metabolomics (study metabolites)	pH, water holding capacity, tenderness, and flavor	Gas chromatography based-metabolomics. Extraction of metabolites in polar and non-polar solvents Liquid chromatography based metabolomics	Sensitive, a large number of compounds can be identified	Only volatile compounds are identified, large molecules weight compounds are not identified, not able to quantify metabolites
	To characterize muscle-specific differences in glycolytic and tricarboxylic acid substrate utilization		Sensitive	
		NMR-based metabolomics	Can determine the concentration, no need to process samples	Less sensitivity
Genomics (the study of DNA)	To determine the genome-wide association of genes associated with tenderness, myoglobin concentration, pHl	Sequence DNA using various platforms such as Illumina. DNA microarray chips help to characterize a large number of genes	Provide useful information about how genes or possible mutation can influence meat quality	Various factors, such as epigenetics and environment, influence outcomes.
Transcriptomics (the study of RNA)	To study RNA expression associated with protein	RNA-seq	Helps to characterize RNA changes associated with meat quality	Need to collect the samples very quickly
Lipidomics (the study of lipids)	To characterize various types of lipids	Extraction of lipids followed by mass spectrometry characterization	Helps to characterize all types of lipids in a biological system	Need bioinformatics expertise to characterize individual fatty acids
Microbiome (characterize all bacteria, fungi, or other microorganisms in meat)	To characterize microorganism by identifying DNA	Extraction of DNA and matching with library	Helps to get a snapshot of all microbes in a biological system	Validation is required.

Fig. 3 Overview of different steps involved in different omics technology

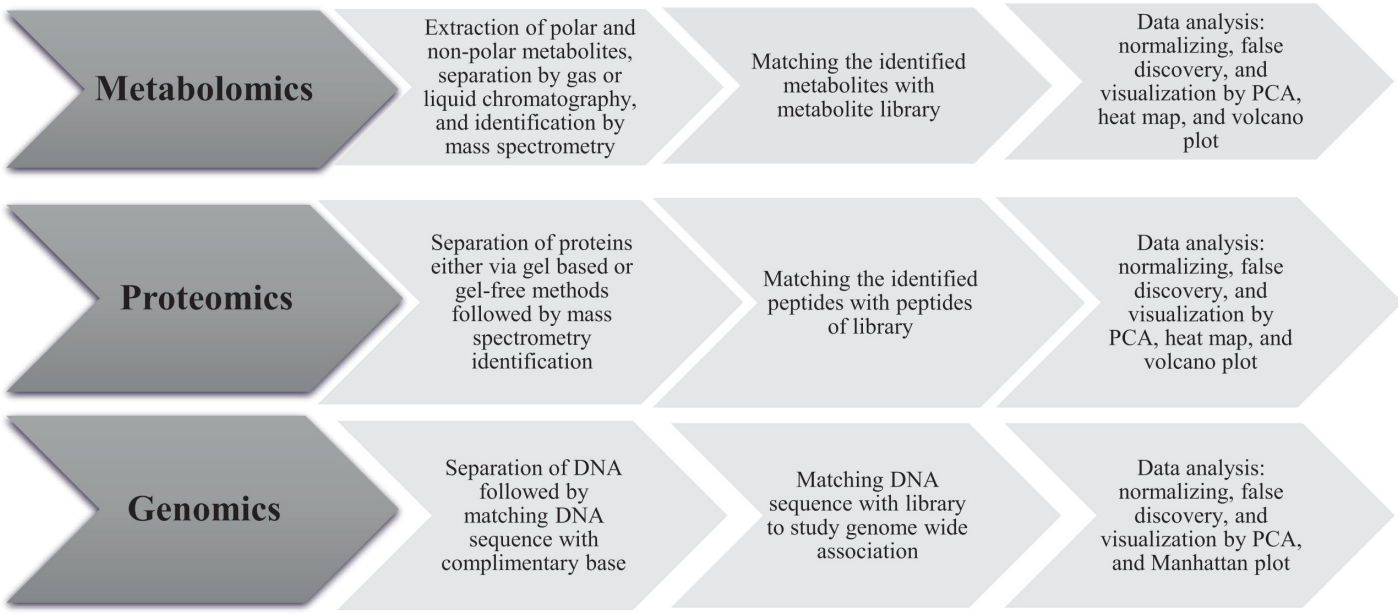

Table 2: Application of omics techniques in meat quiality research

Technique	Application	Reference
Proteomics	Muscle-specific differences in color stability	Joseph *et al.* 2012; Nair, *et al.* 2018a,b
	To understand the biochemical basis of dark-cutting beef	Mahmood, *et al.* 2018
	To determine the impact of aging on meat color	Nair *et al*., 2018ba, b
	To study alteration in purified protein – the impact of lipid oxidation on myoglobin	Naveena *et al*. 2010; Suman, *et al*. 2006
	Effect of aging on tenderness	Nair *et al*. 2019; Paredi *et al*. 2012
	Impact of muscle type on tenderness	Gagaoua, *et al*. 2017
	To study pH decline early postmortem	Kuttappan *et al*. 2017
	To study the effects of proteins on water holding capacity	Marino *et al*. 2014
Metabolomics	Muscle-specific differences in color stability	Abraham *et al*. 2017; Ma *et al*., 2017
	To determine the impact of aging on meat color	Mitacek *et al*., 2019
	To differences in tenderness	D'Alessandro *et al*., 2012
Genomics	To study the impact of gene loci on color	Magalhães *et al*., 2019
	To understand marbling in the meat	Corominas *et al*., 2013
	To study PSE	Strasburg & Chiang, 2009
	To determine the relationship between myoglobin concentration and genomics	Cross *et al*., 2018

METABOLOMICS

Metabolomics is a systematic analysis of small molecules such as amino acids, glycolytic/tricarboxylic intermediates, and fatty acids in a biological system (Fiehn, 2002; Julian, 2004). A real-time snapshot of metabolite changes in a biological system can provide various reactions and helps to identify the ultimate phenotypical changes that happen to cells or tissue due to changes in environment or gene expression. On the other hand, metabolomic analyses involve a study of the entirety of endogenous small molecules (metabolites) within an organism, organ, biological tissue, or cells (Fiehn and Weckwerth, 2003). Metabolomic analysis requires the use of techniques that demand a high level of skill due to the diversity of chemical properties of the metabolites (Straadt *et al*. 2014; Zhang *e t al*.2012).

A comprehensive analysis of metabolome using a single platform may be challenging (Villas-Bôas *et al*. 2005). Metabolomics analysis only quantifies approximately 15-30% of the total metabolites present in a system (Misra *et al*. 2019). Thus, a limited amount of information can be generated from a single omic tool in a stand-alone fashion. The metabolomic analysis involves the usage of either a single platform or a combination of platforms to separate molecules. The popular analytical tools to separate various metabolites are gas chromatography, liquid chromatography, capillary electrophoresis, and nuclear magnetic resonance (Baker *et al*. 2006). The use of mass spectrometry helps to characterize separated molecules. Hence, a combination of gas chromatography and liquid chromatography with mass spectrometry are routinely used in meat science research. Global metabolomics and targeted metabolomics are two types of approaches in metabolomics (Kaddurah-Daouk *et al*. 2008). In the global approach, the analyst tries to identify and characterize all the metabolites present in a biological sample, whereas in the targeted approach, only a specific number or class of metabolites are studied. Both targeted and non-targeted approaches include metabolite separation, detection, quantification, data analysis, and interpretation.

PROTEOMICS

Proteomics is defined as the science of characterizing the entire sets of proteins expressed in a cell or tissue (Bendixen, 2005). The venture into proteomic research was driven by the discovery of post-transcriptional mechanisms (Chevalier, 2010), which revealed that direct measurement of protein expression could provide a useful analysis of biological processes and systems. Therefore, the currently utilized proteomics tool in scientific research usually involves the examination of protein expressions, modifications, or interactions on a large scale (Freeman and Hemby, 2004). For example, functional proteomics with a combination of interactome analysis using electrophoresis, image statistics, and protein sequencing technologies was utilized in identifying particular peptides associated with meat tenderness (Zhao *et al*. 2014).

The quantification of proteins provides useful insights into the role of protein expression changes (up-regulation and down-regulation) in the regulation of cellular activities. Recently, several postmortem studies have deepened our understanding of molecular and cellular mechanisms that regulate meat tenderness (Bjarnadóttir *et al*. 2012; Laville *et al*. 2009; Zhao *et al*. 2014), meat quality (Lametsch and Bendixen, 2001), and meat color (Canto *et al*. 2015; Joseph *et al*. 2012; Maheswarappa *et al*. 2016; Mahmood *et al*. 2018; Nair *et al*. 2016, 2018a, 2018b; Sayd *et al*. 2006). A study by Joseph *et al*. (2012) showed that changes in sarcoplasmic protein expression regulate meat color in beef color stable muscles (*longissimus lumborum*) compared to color labile muscles (*psoas major*). More specifically, they observed a greater abundance of antioxidant protein and chaperones proteins in color stable vs. labile muscles, which may explain the differences in color stability. In addition, Nair *et al*. (2016) demonstrated that sarcoplasmic muscle proteins such as creatine kinase M-type and triosephosphate isomerase are positively correlated with metmyoglobin reducing activity and color stability in color stable compared to color labile muscles. Furthermore, these color stable muscles also show an over-abundance of myofibrillar proteins, including myosin regulatory light chain 2 and myosin light 1/3 (Canto *et al*. 2015).

Tandem mass tag labeling: Although gel-based proteomic approaches have been used to understand the molecular basis of meat quality attributes over the past few years, gel free-approaches are starting to gain more popularity. The gel-free approaches limit some of the drawbacks associated with gel-based approaches such as under-representation of extreme acid/basic proteins and the poor sensitivity for lowly expressed proteins (Nair and Zhai, 2020). Among these, in vitro labeling techniques such as isotope-coded affinity tags (ICAT) and isobaric tags are often used. ICAT uses chemically identical probes with differing mass to tag the treatments, and the peak intensity of the first mass spectra (MS) is used to obtain relative intensities whereas the identity is derived from the second

MS. Isobaric tags such as TMT (Tandem Mass Tag) and iTRAQ (Isobaric Tag for Relative and Absolute Quantitation) enables accurate and multiplexed quantification by using second M.S. (MS/MS) for quantitative analysis (Zhang and Elias, 2017). TMT labeling enables the multiplex of several samples (6-plex to 16-plex) for relative quantitation and increases analytical precision and accuracy. Recently, TMT s labeling in combination with TiO2 phosphopeptide enrichment was used to investigate the postmortem process and to perform a quantitative analysis of protein phosphorylation in ovine muscles with differing color stability (Li *et al*. 2018). These researchers identified 27 phosphoproteins were key color-related proteins, including glycolytic enzymes and myoglobin. Zhai *et al*. (2020) used a TMT labeling approach coupled with high-resolution mass spectrometry to examine proteomic variation between beef L.L. and PM during the early postmortem period and highlighted the potential relationships between metabolism, cell death, and color stability. Moreover,

et al. (2020) identified biomarker candidates for intramuscular fat deposition in pigs using TMT approach.

INTEGRATED OMICS APPROACHES

Integration approaches use a combination of individual omics data tools in a sequential or simultaneous way, bridging the gap from genotypes to phenotypes (Kuo *et al*. 2013). Several researchers utilized omics techniques separately to better understand biochemical changes. However, all biomolecules such as DNA, protein, and metabolite functions are interrelated. Integration is important in understanding the interplay of biological molecules in various biological processes. Thus, despite the exhaustive potential of analysis from a single omics tool, integrating multiple omic tools may further deepen our understanding of postmortem metabolism, and hence could enable us to develop better strategies that will help to develop potential meat quality biomarkers. Therefore, in ideal research, integrated approaches can provide insights about quality issues. The major challenge in integrating all omics is with data processing. Although some software is available, there are limitations in data integration. In recent years, more researchers are integrating different omics techniques to elucidate the molecular basis of quality changes.

The importance of integration of omics data has been realized for a broad range of research areas including; systems microbiology (Fondi and Liò, 2015), food and nutritional science (Kato *et al*. 2011), and disease biology (Pathak and Davé, 2014; Zhang *et al*. 2010). However, successful integration has not yet been realized in meat science research. Additionally, the challenges of integrating omics data depend on variations due to large omic data sets per number of observations. For example, Misra *et al*. (2019) reported that genomes typically have millions of variants, while proteomes and metabolomes include thousands of quantifiable molecules, and thus, differences in the abundance of various fractions make integrated omics data analysis even more complex. However, the promise for the future integration of the omics tool relies on recent advancements in technological platforms for omic data acquisition and data search engines, which will thus further enhance the efficient integration of omic tools.

BIOINFORMATICS TOOLS FOR OMIC DATASETS

Analysis of omics data sets requires some sort of data handling, which is important in addressing issues relating to data filtering and cleaning. Omics data is mainly cleaned by doing transformations, imputations, normalization, and scaling (Armitage *et al*. 2015). However, no clear cut workflows are available for any type of omic data tool. Additionally, different analysis pipelines could yield different results as data workflows are under constant developments. Therefore, it is important always to keep track of the software versions and the name of species used to annotate gene/protein/metabolite for a particular study.

There are various bioinformatics tools and database systems employed in analyzing omics data sets (Berger *et al*. 2013). Differential expression analysis of genes, proteins, and metabolites is one of the widely utilized methods (Misra *et al*. 2019) to identify genes, proteins, and/or metabolites associated with specific biochemical pathways. Most of the tools utilized for proteomics are also extended to genomics data sets. Genomics data sets are analyzed using DNA microarray technologies, also called gene expression profiling (Berger *et al*. 2013).

Proteomics data sets are usually analyzed by employing a multi-approach strategy with a number of software. Peptide spectrums raw data files generated from mass spectrometry are first matched against a downloaded proteome database such as UniPort (http://www.uniprot.org/), NCBI reference sequence (http://www.ncbi.nlm.nih.gov/), etc using MaxQuant (http://www.maxquant.org/). After, the collected raw data files from MaxQuant can then be used for statistical analysis in Perseus (https://omictools.com/perseus-tool) to identify differential expression by analyzing for fold change, T-testing, volcano plots, and hierarchical clustering.

Enrichment of biological functional annotations and gene set enrichment analysis (GSEA) of differentially expressed data sets is usually performed using David (https://david.ncifcrf.org/), and Gprofiler (https://biit.cs.ut.ee/gprofiler/) software, among others freely available online analytical tools. These software map genes to known functional information sources and provide clustering into biological processes, molecular functions, cell compartmentalization, and pathways associated with the differentially expressed proteins/genes. For pathway analysis, Cytoscape (https://cytoscape.org/) is the most widely used platform with various application plugin tools such as Wikipathways (https://www.wikipathways.org/), Reactome pathway (https://www.reactome.org/). In addition, the Kyoto encyclopedia of genes and genomes (KEGG, https://genome.jp/kegg/pathway.html) database also provides a useful pathway annotation analysis. Lastly, the String database (https://www.string-db.org/), a Cytoscape plugin, is important in analyzing potential protein-protein interaction networks associated with the differentially expressed data sets. The ingenuity pathway analysis tool (https://digitalinsights.qiagen.com/) can also be employed for enrichment, pathway, and network analysis.

For metabolomics data sets, LC-MS and GC-MS metabolomics data are usually processed in R packages such as XCMS (http://bioconductor.org/), while NMR metabolomics data processing utilizes Brunker Top Spin software (https://.bruker.com/topspin.html). Several commonly used statistical analyses, including fold change analysis, t-test, volcano plots, principal component analysis (PCA), and metabolite set enrichment (MSEA), can be analyzed in MetaboAnalyst (https://cran.r-project.org/web/packages/MetabolAnalyze/) or metabolomics Galaxy-M (https://github.com/Viant-Metabolomics/Galaxy-M). However, NMR metabolomics post-processing statistical analysis also utilizes a statistical correlation spectroscopy (STOCSY) software designed specifically to identify biomarkers from NMR metabolomics data sets.

CONCLUSION

Omic tools help us to understand the importance of a gene/protein/metabolites in meat quality. The use of multiple omics techniques will significantly increase our understanding of quality changes. A single platform such as gas chromatography-MS, liquid chromatography-MS, or NMR cannot demonstrate all metabolites. Hence, depending on the objective of the research, an appropriate selection of techniques is critical. Furthermore, data analysis and interpretation remain a significant challenge. The commonly available software help in analyzing data, but the lack of consistency in various parameters can influence the outcome. Johanningsmeier *et al*. (2016) indicated that omics and related disciplines are often referred to as hypothesis-generating as these technologies can demonstrate changes in several biomolecules at a particular set of conditions. A systematic analysis is critical to validate the results of omics techniques. A combination of all omics techniques will improve our understanding of postmortem changes and will help the processors to adopt strategies to improve meat quality and develop biomarkers.

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