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<td>Author</td>
<td>Putri Widyanti Harlina*, Vevi Maritha*, Ida Musfiroh*, Syamsul Huda*, Nandi Sukri*, Muchtaridi Muchtaridi**</td>
</tr>
<tr>
<td>Affiliation</td>
<td>1. Department of Food Industrial Technology, Faculty of Agro-Industrial Technology, Universitas Padjadjaran, 45363 Bandung, Indonesia. 2. Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, 45363 Bandung, Indonesia.</td>
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<td>Special remarks – if authors have additional information to inform the editorial office</td>
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<tr>
<td>ORCID (All authors must have ORCID)</td>
<td>Putri Widyanti Harlina (<a href="http://orcid.org/0000-0002-9504-7252">http://orcid.org/0000-0002-9504-7252</a>) \ Vevi Maritha (<a href="http://orcid.org/0000-0002-3697-7513">http://orcid.org/0000-0002-3697-7513</a>) \ Ida Musfiroh (<a href="https://orcid.org/0000-0002-2569-8914">https://orcid.org/0000-0002-2569-8914</a>) \ Syamsul Huda (<a href="https://orcid.org/0000-0002-8160-111X">https://orcid.org/0000-0002-8160-111X</a>) \ Nandi Sukri (<a href="https://orcid.org/0000-0002-3850-3856">https://orcid.org/0000-0002-3850-3856</a>) \ Muchtaridi Muchtaridi (<a href="https://orcid.org/0000-0002-6156-8025">https://orcid.org/0000-0002-6156-8025</a>)</td>
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**CORRESPONDING AUTHOR CONTACT INFORMATION**

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<th>For the corresponding author (responsible for correspondence, proofreading, and reprints)</th>
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<tr>
<td>First name, middle initial, last name</td>
<td>Putri Widyanti Harlina; Muchtaridi Muchtaridi</td>
</tr>
<tr>
<td>Email address – this is where your proofs will be sent</td>
<td><a href="mailto:putri.w.harlina@unpad.ac.id">putri.w.harlina@unpad.ac.id</a> ; <a href="mailto:muchtaridi@unpad.ac.id">muchtaridi@unpad.ac.id</a></td>
</tr>
<tr>
<td>Secondary Email address</td>
<td><a href="mailto:harlinafoodtech2020@gmail.com">harlinafoodtech2020@gmail.com</a></td>
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| Postal address | 1. Department of Food Industrial Technology, Faculty of Agro-Industrial Technology, Universitas Padjadjaran, 45363 Bandung, Indonesia.  
2. Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, 45363 Bandung, Indonesia. |
| Cell phone number | +6281316889866 ; +6281394495569 |
| Office phone number | +62-22-7798844 |
| Fax number | - |
Title: Possibilities of LC-MS-based metabolomics and lipidomics in the authentication of meat products: A mini review

Running Title: Metabolomics and lipidomics in authentication of meat product

Abstract

The LC-MS (Liquid Chromatography Mass Spectrometry)-based metabolomic and lipidomic methodology has great sensitivity and can describe the fingerprint of metabolites and lipids in pork and beef. This approach is commonly used to identify and characterize small molecules such as metabolites and lipids, in meat products with high accuracy. Since the metabolites and lipids can be used as markers for many properties of a food, they can provide further evidence of the foods authenticity claim. Chromatography coupled to mass spectrometry is used to separate lipids and metabolites from meat samples. The research data usually is compared to lipid and metabolite databases and evaluated using multivariate statistics. LC-MS instruments directly connected to the metabolite and lipid databases software can be used to assess the authenticity of meat products. LC-MS has good selectivity and sensitivity for metabolomic and lipidomic analysis. This review highlighted the combination of metabolomics and lipidomics can be used as a reference for analyzing authentication meat products.

Keywords: Meat products; Metabolomics; Lipidomics; Authentication; LC-MS.
Introduction

Meat and meat products are significant sources of nutrition for humans, including proteins, lipids, minerals, and vitamins. Every year, beef consumption rises, and one consequence of this rise is the mixing of beef with other meats, such as pork, during processing. Furthermore, meat adulteration may violate religious beliefs; for example, Kosher and Halal food laws prohibit the consumption of pig or pork-related items (Lim and Ahmed, 2016; Alzeer et al., 2018). Many strategies have been implemented to ensure the authenticity of meat and meat products (Abbas et al., 2018; Mahbubi et al., 2019) but still the coverage is insufficient, and certification of all meat products for protection against adulteration is unfeasible. As a result, effective methods are necessary for assuring the meat industry's proper development, and rapid, comprehensive, accurate, and reliable detection technologies are crucial to achieving this goal. One approach for guaranteeing food authenticity could be metabolomic and lipidomic technology (Sheikha et al., 2017; Ali et al., 2020; Vanany et al., 2020; Islam et al., 2021) compared to existing methods such as PCR (Polymerase Chain Reaction), which has the limitation of being easily degraded in processed foods so that it has the potential to cause false negatives (Lubis et al., 2016; Jannat et al., 2018; Jannat et al., 2020). Metabolomic as method with high accuracy, comprehensive analysis of the whole metabolome which refer to the full complement of small molecule, while lipidomic explored area within lipid analytics and more specifically meat adulteration, so this method can used authentication of meat product (Trivedi et al., 2016; Emwas et al., 2019).

Metabolomics is a method of qualitative and quantitative analysis of metabolites in cells, tissues and biological fluids with small molecular weights, 100 to 1,000. Metabolites are the result of gene expression derives from the interaction between the
Metabolites consist of an intermediate compound and metabolism product. The fingerprint characteristics of metabolites found in meat, such as amino acids, sugars, organic acids, nucleic acids, and their derivatives, could be provided through metabolomics. With minimal sample preparation, this approach can examine the components globally (De Paepe et al., 2018). The metabolomics approach to pork and beef study could provide a picture of the metabolites present in both foods. The metabolite profile of pork differs greatly from that of beef, hence the latter's metabolite profile could be used as a baseline for determining the meat's authenticity.

Metabolomic and lipidomic can also distinguish pork mixture in mutton and chicken by looking at the different metabolites and lipid profiles (Wang et al., 2020). Lipidomics could be another way to look into the existence of pork mixture in meat products (Castro-Puyana et al., 2017; Yang et al., 2019).

Lipidomics is a comparatively new field of study, and it is developing quickly because to recent advancements in data analysis, bioinformatics data processing, and system biology techniques that are connected to other omics systems (Kliman et al., 2011). Various types of lipids, such as fatty acids and triglycerides, are found in the metabolome and the most distinctive biomarkers in which each type of tissue in meat has a different lipid profile, making it possible to identify unwanted species in a food product. For each animal species, there are a number of fatty acids located in specific tissue that can be used to differentiate between the various other animal species found in meat products. However, another significant benefit of lipidomic research is that it enables for the identification of animal species based on their lipid profiles (Dettmer et al., 2007; Ballin, 2010; Domínguez et al., 2019). Pork has a completely different lipid profile compare than beef. To evaluate whether a product contains pork or beef,
the lipid profile of pork and beef can be utilized as a guideline. LC-MS (Liquid Chromatography Mass Spectrometry) could be used in metabolomic and lipidomic techniques to investigate the authentication of meat.

The LC-MS method that integrates metabolomic and lipidomic analysis in pork and beef is a new technology with excellent sensitivity and provides the fingerprint of metabolites and lipids in biological samples. Organic chemicals and some inorganic substances can be analyzed using LC-MS (Gorrochategui et al., 2016). Sample preparation, data acquisition, and subsequent processing could be made easier using a mix of chromatographic and mass spectrometry techniques (Moosmang et al., 2019). This approach is frequently used to determine and characterize tiny molecules in meat products with high separation, such as metabolites and lipids. One of the advantages of LC-MS is the simplicity with which samples can be prepared. Mass spectrometry could verify that the types of metabolites and lipids present in the sample. Another benefit of LC-MS in metabolomic and lipidomic research is that it can identify all types of metabolites and lipids in a single sample run. This makes both analyses very efficient while using LC-MS (Neef et al., 2020).

Based on the preceding description, more investigation is necessary to answer the question of whether metabolite and lipid profiles can be utilized to determine the authentication validity of meat. To answer this question, a systematic review involving a comprehensive metabolomic and lipidomic approach is required, and it may be able to provide a comprehensive reference in the assessment of meat products (Rohman and Che Man, 2011; Demirhan et al., 2012; Mostafa, 2020; Pranata et al., 2021).
Analytical methods for authentication of meat products

Authenticity detection technologies for meat and meat products, such as PCR based on deoxyribonucleic acids (DNAs), protein technologies, and spectroscopic technologies based on specific metabolites, have all been developed in the previous two decades (Li et al., 2020). Presently, PCR (Amaral et al., 2017) and proteomics methods are routinely used for the species authentication (Von Bargen et al., 2014).

PCR is the most extensively used method for determining of meat products based on the presence of DNA, whereas proteomic is a method for determining of meat products based on their protein profile (Nakyinsige et al., 2012). Furthermore, PCR may detect pork DNA in a product (Nakyinsige et al., 2012; Izadpanah et al., 2017; Yuswan et al., 2018) and can detect a very small number of DNA copies. The hybridization of particular oligonucleotides to the target DNA and the synthesis of millions of copies flanked by these primers are the foundations of PCR amplification. Amplification of DNA fragments followed by agarose gel electrophoresis for fragment size verification is the most basic PCR approach for determining the presence of any species in meat products. Appropriate genetic markers are chosen to create the examination in order to properly detect species by PCR (Izadpanah et al., 2017). Porcine gelatin of pork can also be employed as an indicator of meat products in PCR analysis. The presence of DNA porcine gelatin of pork in the sample can be determined using the PCR technique (Rohman et al., 2020). The proteomic technique is another method for determining the validity of meat products. The goal is to determine the validity of meat products by examining for proteins, biological activity, post-translational modifications, and interactions in cells, as well as identifying the proteome in response to changes in porcine biological circumstances in the samples (Zamaratskaia and Li, 2017). LC-QTOF-MS (Liquid Chromatography Quadrupole
Time of Flight Mass Spectrometry) is a method for determining the type of meat using a powerful tool for identifying protein peptides (Sarah et al., 2016; Zamaratskaia and Li, 2017). Protein extraction precedes MS (Mass Spectrometry) or LC-QTOF-MS analysis in the proteomic analysis method. In proteomics, mass spectrometry is the most typical approach for detecting proteins or peptides. This method has a wide range of applications, including meat science research, but it is hampered by the large biochemical heterogeneity of proteins and the inability to detect low protein levels. The detection of meat products using a proteomic technique has a high selectivity because only certain types of pork peptides can be found (Stachniuk et al., 2021). The PCR and proteomics methods both have their own set of difference when it comes to detecting adulteration in meat and meat products, Therefore, recent advances of the omics technologies (particularly metabolomics and lipidomics) are comprehensively discussed in this review.

**Beef Meat and Its Products**

Meat and its products are consumed widely throughout the world as a source of high-quality protein, essential amino acids, vitamins, and necessary minerals (Demirhan et al., 2012). A suitable analytical technique, such as HS-SPME/GC–MS (Headspace Solid-Phase Micro Extraction/Gas Chromatography-Mass Spectrometry), which employs volatile compounds to identify the meat authenticity, is used to ensure the authentication of beef and its products. The presence of alcohol compounds, 2-butanol and 1-octen-3-ol in a mixture of beef and pork can be used as a reference. These chemicals indicate presence of a pork mixture in meat products (Pavlidis et al., 2019; Hossain et al., 2020) Another method to ensure the meat authenticity is by EvaGreen real-time PCR. This validated method is able to detect pork DNA specifically in meat product samples. This method can detect 0.01-100% pork
contamination in beef meatballs with high accuracy and precision. In addition to the two procedures mentioned above, other methods such as FTIR (Fourier-Transform Infrared Spectroscopy) and LC-MS can be used to determine the authenticity of beef and its products (Lubis et al., 2016; Yuswan et al., 2018) FTIR also can be used to assess the meat authenticity. This approach can identify functional groups in proteins as pig identifiers, allowing pork-containing products to be recognized (Lubis et al., 2016). By looking at the peptide fingerprints found in pork, LC-MS can be utilized to detect the meat authenticity. Moreover, chemometric technique also used to assess the type of peptide present in pork using this peptide fingerprint. Pork marker peptide is the result of this chemometric analysis, and it is utilized to determine the meat authenticity (Yuswan et al., 2018).

Chicken Meat and Its Products

Chicken meat is one of the meat products that provide the body with essential amino acids, fatty acids, and vitamins that the body need (Ali et al., 2019). The analytical methods are needed to verify that chicken meat authenticity. The high-sensitivity technology for detecting the presence of a pork mixture in the product is necessary in this case. LC-QTOF-MS/MS is the analytical method for analyzing chicken meat and its products. The protein acquired from the MS spectra is matched to the absolute protein expression (APEX) database using the proteomic principle. The authenticity of chicken meat products can be determined using a peptide derived from other meat such as pork (Montowska and Fornal, 2017). Another method to examine the authenticity of chicken meat and its products is proteomic analysis using MALDI TOF/TOF (Matrix-Assisted Laser Desorption/Ionization-Time of Flight). Determination of halal chicken meat is not only based on the presence of a mixture of pork but also the method of slaughter. In this method, it is possible to detect the
proteome of chicken meat slaughtered in a halal and non-halal way. Beta-enolase, pyruvate kinase, and creatine kinase compounds are the ones that could have higher levels when slaughtered in an illegal manner (Salwani et al., 2015).

**Animal Fat Products**

There is a growing need for animal fat nowadays, based on the Statistic Government Bureau in Indonesia, export value of animal and vegetable oil fats has increased from 19,329 million USD in 2018 to 19,709,5 million USD in 2020 (Qodri, 2018). Animal fat is essential product that has various purposes in the body, including providing energy and forming adipose tissue. Fat is the most energy-dense food, producing 9 kcal per gram, 2.5 times the energy provided by carbohydrates and protein in the same quantity. Fat can produce fatty acids and cholesterol needed to form cell membranes in all organs.

Halal meat products are important since consumption of halal meat might influence one's attitude towards halal slaughter (Jalil et al., 2018). To be declared halal, meat products must meet a number of conditions relating to their preparation, condition for analysis such as towing before analysis, and composition. Consumers may trust halal meat labeling to ensure that the meat is of good quality, high value, safe, animal-friendly, and environmentally friendly (Haleem et al., 2021; Lim et al., 2022) Animal fats such as chicken and beef fat are permissible (Sin et al., 2019), however pork fat is prohibited for Moslem according to Shariah (Islamic law) (Ahmad et al., 2018).

Beef fat is one type of meat products that is usually consumed nowadays. Beef is abundant in fat and contains important nutrients such as essential fatty acids, in addition to protein. Consumers’ concerns and awareness about the eating of high-fat meat items have an impact on meat consumption patterns (Mahbubi et al., 2019). The
following is a representation of beef fat content: saturated fatty acids, n-6 polyunsaturated fatty acids, n-3 polyunsaturated fatty acids, and trans fatty acids are the different types of fatty acids. Fatty acids in beef vary based on genotype, muscle type, and feeding methods in general. Long-chain n-3 and n-6 polyunsaturated fatty acids found in beef provide extra health benefits, including improved maternal and child health, growth and development, and cognitive function and psychological state in humans (Troy et al., 2016). Animal fat can be investigated from the use of technology including spectroscopy and chromatography (Rohman and Fadzillah, 2021). Raman spectroscopy is one of the spectrophotometric methods that can be used to identify the authenticity of animal fats (Lee et al., 2018). The resulting spectra could be forwarded using various types of databases in this manner, allowing them to determine the types of unsaturated and saturated fatty acids. The types of fat are not only qualitatively but also quantitatively examined in this method. It could be possible to accurately detect the type and amount of pork animal fat. Another method of authenticity analysis of animal fat by chromatographic method is using HPLC-NMR (High-Performance Liquid Chromatography Nuclear Magnetic Resonance) and GC-MS (Gas Chromatography–Mass Spectrometry). HPLC-NMR is able to separate well the lard compounds in the sample which is then followed by reading the structure of the compound. Pork fat has the unique characteristic of containing polyunsaturated fatty acids. This fat could be used as a target to determine the authenticity of animal fat. Determination of the authenticity of animal fats can also be conducted with a targeted metabolomic approach using GC-MS, a simple method with good separation technique (Fadzillah et al., 2017; Heidari et al., 2020) Methyl myristate, methyl palmitate, methyl oleate, and methyl stearate are examples of targeted metabolites of lard that can be analyzed in the samples.
It is critical to determine authentication of meat. In order to acquire valid results that may be used to declare authenticity, new methods are still being developed. By examining the metabolite profile, one way for determining the authenticity of meat and its products is metabolomics. Metabolomic studies can use either spectrophotometry or chromatography (Muroya et al., 2020). The spectrophotometry used in metabolomic analysis is Ultraviolet-Visible, Infrared, Raman, and Nuclear Magnetic Resonance combined with chemometrics for spectral data. MS (Junot et al., 2014) and non-MS such as Nuclear Magnetic Resonance (NMR) are the most widely used methods (Consonni and Cagiani, 2019). In addition, different types of separation techniques are incorporated in most MS-based, depending on the lipophilicity and polarity of the desired metabolite. Combined with statistical analysis, multivariate analysis, and bioinformatics databases, metabolomics provides for finding biomarkers (Sugimoto et al., 2012).

The most widely utilized multivariate analyses in certified meat products are Principal Component Analysis (PCA), Partial Least-Squares Discriminant Analysis (PLS-DA), and Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) (Dailey, 2017). PCA is used to narrow down the list of metabolites and lipids to only the most important ones (Zhang et al., 2022). Other animal species that are present in meat can be identified by PCA when validating LC-MS data (Kang et al., 2022). Typically, Minitab, Orange, R Studio, and Unscramble are used for PCA analysis. The PLS-DA classification is a good choice for recognizing meat products. This is because the PLS-DA approach, whose implementation is exceedingly user-friendly and is extensively utilized in the most well-known statistical software
packages like R Studio, is also quite popular. Additionally, PLS-capacity DA’s to analyze highly linear and noisy data is one of its advantages (Utpott et al., 2022).

Using R Studio software, OPLS-DA provides a quick, easy, and effective multivariate analysis. In order to find meat-specific quantitative peptides created using liquid chromatography-tandem mass spectrometry (LC-MS/MS), OPLS-DA was applied to halal analysis. To choose species-specific peptides that significantly assist in classification, the OPLS-DA model was developed. Three distinct quantitative peptides were found in products with various beef proportions after the statistical process flow. LC-MS/MS was used to build quantitative methodologies for the specific quantitative peptides selected. Commercial beef products were subjected to quantitative results. The devised method is extremely precise, repeatable, and sensitive. According to Kang et al. (2022), LC-MS/MS integration with OPLS-DA is an efficient method to screen for particular quantitative peptides and certify beef product.

Apart from spectrophotometry, chromatography is also used in metabolic analysis. In metabolomic analysis, the use of LC-MS is critical, and one example is the use of UHPLC-QTOF (Ultra-High Performance Liquid Chromatography Quadrupole Time-of-Flight) with REIMS (Rapid Evaporative Ionization Mass Spectrometry), which can provide metabolites in meat (Wang et al., 2020). The workflow of metabolomics analysis in meat samples can be seen in Fig. 1. Besides metabolomics, lipidomics is another way for determining the validity of meat products. The result of metabolite analysis can be used for authentication meat products such as myosin-2 (Yuswan et al., 2018), 3-Oxohexane acid glycerides, arabitol, creatinine, glycine and phosphate (Trivedi et al., 2016). Also the lipid component that can be used for
authentication such as sphingomyelins, cerebrosides, globosides, gangliosides or sulfatides (Trivedi et al., 2016).

The workflow summarizing the different steps in lipidomics analysis can be seen in Fig. 2. The study of lipid profiles that can be applied to meat products in order to assess authenticity is known as lipidomics. Pork's lipid profile is undoubtedly different from that of other meats, and this unique lipid profile can be used to confirm the meat authenticity. Lipidomic analysis to determine meat authenticity can use LC-MS, one of which uses LC-ESI-MS/MS (Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometric). This method is able to interpret the lipid profile combined with MS data. The lipid profile of pork-containing meat products can be identified well (Dirong et al., 2021).

The information on the compounds resulting from LC-MS in lipidomic and metabolomic study is enormous, reaching in the thousands. The metabolites that have been detected are then determined for the identified compounds using the databased compound discover, and the profile of lipids and sub-lipids is determined using the databased LipidSearch (Korf et al., 2019; Medema and Fischbach, 2015). Due to the size of the database's results for metabolites and lipids, multivariate analysis is necessary to identify the metabolites and lipids that will serve as meat products' authenticity indicators (Trivedi et al., 2016).

**Metabolomic Studies**

Metabolomics is the study of the profile of low molecular weight metabolites. The metabolomics approach can be applied to determine the authenticity of meat. The metabolite profile in pork could certainly be different from that of beef or other meats. For example, research by Rocchetti et al. (2020) was able to identify metabolites in pigs with UHPLC-QTOF-MS (Ultra-High Performance Liquid Chromatography Quasi-ToF Mass Spectrometry)
Chromatography-Quadrupole Time-of-Flight Mass Spectrometry) which obtained hexanoylcarnitine, 4-hydroxy-2-nonenal, 6-hydroxypentadecanedioic acid, 9S, 11S, 15S, 20-tetrahydroxy-5Z, 13E-prostadienoic acid (20-hydroxy-PGF2a), sativa acid, and glycerophospholipid. Moreover, Ali et al. (2020) was able to identify glucose, amino acid, inosine, hypoxanthine, and arginine in broiler chickens slaughtered in an illegal manner using UHPLC-QTOF-MS. Furthermore, Jia et al. (2021) mentioned that 103 metabolites could be identified such as L-phenylalanine, L-isoleucine, L-histidine, guanosine, guanine, creatinine, glutathione, and nicotinic acid in goat meat using UHPLC-QTOF-MS. Several studies have reported the use of highly accurate metabolomic technologies to evaluate metabolite profiles, indicating that an approach based on this method could be used to determine qualities in the meat.

Metabolomics is a method for determining the numerous metabolite profile found in pork and beef. The qualities of the metabolite profiles in pork and beef could be distinguished by using these metabolites. Chromatography in mass spectrometry is used to separate metabolites from samples. UPLC (Ultra Performance Liquid Chromatography) is usually being used to separate metabolites because of its ability to separate well. The TOF-MS (Time of Flight-Mass Spectrometry) apparatus could be coupled to the UPLC device, making it easier to get the research data. The UPLC-TOF-MS (Ultra Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry) for metabolomic analysis was also described by Jia et al. (2021) and identified 103 metabolite profiles in goat meat. The UPLC-TOF-MS method is an effective way to figure out the metabolite profiles of meat samples. The data could be compared to the Compound Discovered database, and multivariate statistics could be used to interpret the results. Pork's metabolite profile, which distinguishes it from beef, could be utilized as a standard for
determining authenticity in food samples. Metabolites such as decanoylcholine, glycyl-lysine, and oleic acid can be used to authenticate meat products. The resume authentication of meat product using metabolomics can be shown in Table 1. Moreover, the metabolite extraction method was according to Jang et al. (2019), sample was mixed with 150 µL methanol: acetonitrile: water (40: 40: 20, extraction solvent), vortexed, and immediately centrifuged at 16,000 g for 10 min at 4°C. The supernatant was collected for analysis.

Lipidomic Studies

Lipidomics is a novel branch of research that examines the structure and function of lipids produced in plant and animal cells, as well as their interactions with other lipids, metabolites, and proteins (Li and Guo, 2017) can be regarded as a relatively unexplored area in food analysis (Aiello et al., 2011) and more specifically on adulteration of meat. Using the LC-MS approach, Mi et al. (2019) were able to successfully detect the component of lipids in several species of pork in China. They results showed that 61 types of glycerolipids, 17 glycerol phospholipids, 4 sterol lipids, 2 sphingolipids, 3 polyketides, 7 fatty acids, and 6 phenol lipids. Trivedi et al. (2016) used GC-MS and UHPLC-MS to evaluate lipidomic profiles of beef contaminated with pork. They found lipid components in beef, such as sphingomyelins, cerebrosides, globosides, ganglioside or sulfatides. Furthermore, Artegoitia et al. (2019) investigated the metabolomic and lipidomic profiles of beef fed efficiency feed using the UPLC-QTOF-MS technique, the results showed there were 20 types of phospholipids and cholesterol, such as phosphatidylcholine, phosphatidylethanolamine, lysophosphatidylcholine, and lysophosphatidylethanolamine. These studies have proven that the lipidomic method may be used to examine lipids in pork and beef utilizing chromatographic
techniques to separate lipids for further examination. The type of chromatography
used in previous study was column chromatography. With a single run, column
chromatography may separate a large number of organic molecules in a short amount
of time. Several scientific publications have reported the use of very accurate
lipidomic technologies to examine lipid profiles, indicating that this technology could
be used to determine meat authenticity, does not containing other meat such as pork.
Glyserolipid and spingolipid are examples of lipids that can be used to authenticate
meat.

In addition, the sample pretreatment for lipid and metabolite extractions are using
similar methods in a different mixture. The lipid from meat were extracted according
to the method of Harlina et al. (2021), whereas, meat sample were homogenizer in a
mixture of chloroform: methanol: distilled water (120: 120: 60, v/v/v) at 11000 g
using a homogenizer for 2 min. Then, the homogenized mixture was treated with
ultrasound (20ºC, 80% power, 30 min). The mixture was filtered through a Buncher
filter funnel. The chloroform phase (bottom phase) was drained off into an
Erlenmeyer flask. The lipid in chloroform was decanted into a round-bottom flask
through a filter paper. Before it was evaporated at 55ºC using rotary evaporator and
the residual solvent were removed by flushing with nitrogen. The lipid was stored at
-20ºC until was analyzed.

Primary lipids such as cholesterol and its esters, as well as triglycerides, are found
in nonpolar lipids, lipids are compounds that are soluble in nonpolar solvent such as
chloroform (Han and Gross, 2005; Yu et al., 2020). Phospholipids, sphingolipids,
rhamnolipids, and glycolipids are the most common lipid classes found in polar lipids.
Furthermore, phospholipids are divided into numerous groups based on the phosphate
classes, including such as phosphatidylcholine (PC), phosphatidylethanolamine (PE),
phosphatidylinositol (PI), phosphatidylglycerol, phosphatidylserine (PS), and phosphatidic acid (Li and Guo, 2017). Glycerophospholipid categories in the meat can be seen in Fig. 3. PC, PE, PI, and PS are the major glycerophospholipids found in the membrane. They have various functions in the plasma membrane's exoplasmic and cytoplasmic functions, and they provide a semi-permeable barrier to keep the cell intact (Arish et al., 2015).

Lipidomics is a technique for determining the numerous lipid species found in the food samples. According to Harlina et al. (2021), depending on the lipid species and head groups, lipids can be identified using positive or negative ions. The majority of phospholipids are found in both positive and negative ion modes as distinct adducts, including +H+, +NH₄+ in positive mode and -H, +CH₃COO, or +HCOO- in negative mode. Triglycerides and diglycerides are examples of neutral lipids that are all recognized in positive mode as NH₄ adducts. However, only negative ion mode can identify the fatty acid contents of phospholipids, while positive ion mode can declare head group and/or neutral loss. Therefore, lipids are amphiphilic substances that ionize in both positive and negative modes. The MS based shotgun of lipidomics can be seen in Fig. 4.

Resume authentication of meat product using lipidomic can be shown in Table 2. UPLC is currently used to separate lipids since it can separate up to the sub-class of lipids. QE-HESI (Q-Extractive Heated Electrospray Ionization) can be coupled to the UPLC equipment, making it easier to get research data. Rivas and Zhang (2016) described the use of the UPLC-QE-HESI (Ultra Performance Liquid Chromatography Q-Extractive Heated Electrospray Ionization) for lipidomic analysis. This method has good selectivity and accuracy for determination lipids in sample (Narváez-Rivas and Zhang, 2016). They used this equipment to detect 430 lipid profiles in plasma. The
UPLC-QE-HESI is an excellent tool for determining the lipid profiles of meat samples. The research data can be compared to lipid databases evaluated using multivariate statistics. Pork's lipid profile, which distinguishes it from beef, could be utilized to determine authenticity in food samples (Narváez-Rivas and Zhang, 2016; Holčapek et al., 2018). The advantages and disadvantages of metabolomic and lipidomic for authentication in meat products can be seen in Table 3.

**Future Perspective**

The techniques of metabolomics and lipidomics can be used to identify meat products. The combination of the two will give a clearer picture of the meat profile (Wang et al., 2021). The complete lipid and metabolite profiles can effectively distinguish between meat varieties (Wang et al., 2021; Wu et al., 2021; Zhang et al., 2021). In the identification of meat products, combining metabolomic and lipidomic approaches could provide a more comprehensive overview (Ellis et al., 2016; Munekata et al., 2021). The combination of these two methods can be used to determine the authentication of meat products, by looking at the metabolites and lipids in meat products whether they contain pork (D’Alessandro and Zolla, 2013). In comparison to the metabolomic or lipidomic method alone, the combination of these two procedures could precisely evaluate the authentication of meat products (Chin et al., 2009; Picó, 2015; Capozzi et al., 2017; Yuliana et al., 2021).

**Conclusion**

Metabolomics is the study of metabolite profiles that can be used to identify the authenticity of meat products by examining metabolite profiles in pork that are not represented by beef. Lipidomics is a lipid profile analysis that may be used to determine the authenticity of meat products by examining the lipid profile of pork, which beef excludes. LC-MS instruments such as the UHPLC-QTOF-MS and
UPLC-QE-HESI, which are directly connected to the metabolite and lipid databases software, can be used for metabolomic and lipidomic approaches to assess the authenticity of meat products. Combination metabolomic and lipidomic approaches to assess the authenticity of meat products would be more accurate because it can describe extensive lipid and metabolite profiles in meat.

**Conflicts of Interest**

The authors have declared no conflicts of interest.

**Acknowledgments**

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**References**


issues in two countries with predominantly Muslim and non-Muslim populations. PLOS ONE 13(10):0204094.


**Figure Captions**

**Fig. 1.** Workflow for metabolomics analysis in meat samples

**Fig. 2.** Workflow summarizing the different steps in lipidomics analysis

**Fig. 3.** Glycerophospholipid categories in the meat

**Fig. 4.** MS based shotgun lipidomic
<table>
<thead>
<tr>
<th>No</th>
<th>Title</th>
<th>Refs</th>
<th>Years</th>
<th>Objectives</th>
<th>Equipment</th>
<th>Metabolite Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1H-NMR-based Metabolomic Profiling and Taste of Stewed Pork-Hock in Soy Sauce. LC–QTOF-MS identification of porcine-specific peptide in heat treated pork identifies candidate markers for meat species determination</td>
<td>(Yang et al., 2019)</td>
<td>2019</td>
<td>Steward pork</td>
<td>1H-NMR</td>
<td>Amino acids, sucrose, β-glucose, acetate and creatinine</td>
</tr>
<tr>
<td>2</td>
<td>A volatilomics approach for off-line discrimination of minced beef and pork meat and their admixture using HS-SPME GC/MS in tandem with multivariate data analysis</td>
<td>(Sarah et al., 2016)</td>
<td>2016</td>
<td>Meat (pork, beef, chicken and chevon)</td>
<td>LC-QTOF-MS</td>
<td>Seven porcine-specific peptides, two were derived from lactate dehydrogenase, one from creatine kinase, and four from serum albumin protein</td>
</tr>
<tr>
<td>3</td>
<td>A volatilomics approach for off-line discrimination of minced beef and pork meat and their admixture using HS-SPME GC/MS in tandem with multivariate data analysis</td>
<td>(Pavlidis et al., 2019)</td>
<td>2019</td>
<td>Meat (beef and pork)</td>
<td>GC-MS</td>
<td>Alcohols, 2-butanol and 1-octen-3-ol</td>
</tr>
<tr>
<td>4</td>
<td>Chemometrics-Assisted Shotgun Proteomics for Establishment of Potential Peptide Markers of Non-Halal Pork (Sus scrofa) among Halal Beef and Chicken Discrimination between vegetable oil and animal fat by a metabolomics approach using gas chromatography–mass spectrometry combined with chemometrics</td>
<td>(Yuswan et al., 2018)</td>
<td>2018</td>
<td>Meat (beef, chicken and pork)</td>
<td>LC-MS</td>
<td>7 peptides marker</td>
</tr>
<tr>
<td>5</td>
<td>Chemometrics-Assisted Shotgun Proteomics for Establishment of Potential Peptide Markers of Non-Halal Pork (Sus scrofa) among Halal Beef and Chicken Discrimination between vegetable oil and animal fat by a metabolomics approach using gas chromatography–mass spectrometry combined with chemometrics</td>
<td>(Heidari et al., 2020)</td>
<td>2020</td>
<td>Animal Fats</td>
<td>GC-MS</td>
<td>Methyl myristate, methyl palmitate, methyl oleate, and methyl stearate</td>
</tr>
</tbody>
</table>

Impact of a Pitanga Leaf Extract to Prevent Lipid Oxidation Processes during Shelf Life of Packaged Pork Burgers: An Untargeted Metabolomic Approach (Rocchetti et al., 2020).


The Metabolites: An Integrated metabolite Profiling and Lipidomics Approach For The Detection of The Adulteration of Beef With Pork (Trivedi et al., 2016).

Hexanoylcarnitine, 4-hydroxy-2-nonenal, 6-hydroxypentadecanedioic acid, 9S,11S,15S,20-tetrahydroxy-5Z,13E-prostadienoic acid (20-hydroxy-PGF2a), sativic acid.

Histidin, inosin, hypoxantine.

Arabitol, citric acid, glucose 6-phosphat, glycine, malic acid.

Note: 1H-NMR (Hydrogen-1 Nuclear Magnetic Resonance); LC-QTOF-MS (Liquid Chromatography Quadrupole Time of Flight Mass Spectrometry); GC-MS (Gas Chromatography-Mass Spectrometry); LC-MS (Liquid Chromatography-Mass Spectrometry); UHPLC-QTOF-MS (Ultra-High Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry); UHPLC-MS (Ultra-High Performance Liquid Chromatography-Mass Spectrometry).
### Table 2. Lipidomic approaches for authentication of meat product

<table>
<thead>
<tr>
<th>No</th>
<th>Title</th>
<th>Refs</th>
<th>Years</th>
<th>Objectives</th>
<th>Equipment</th>
<th>Lipids Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Authentication of butter from lard adulteration using high-resolution of nuclear magnetic resonance spectroscopy and high-performance liquid chromatography</td>
<td>(Fadzillah et al., 2017)</td>
<td>2017</td>
<td>Lard</td>
<td>NMR, HPLC</td>
<td>Triacylglycerol and fatty acids</td>
</tr>
<tr>
<td>2</td>
<td>Quantitative analysis of lard in animal fat mixture using visible Raman spectroscopy</td>
<td>(Lee et al., 2018)</td>
<td>2018</td>
<td>Lard</td>
<td>Raman Spectroscopy</td>
<td>Quantitative fat oil</td>
</tr>
<tr>
<td>3</td>
<td>Characterization and Discrimination Of Selected China’s Domestic Pork Using an LC-MS-based Lipidomic Approach</td>
<td>(Wang et al., 2020)</td>
<td>2020</td>
<td>Raw pork meat</td>
<td>UHPLC-QTOF-MS</td>
<td>Multiple triglyceride (TG), diacylglycerol (DG), and PL</td>
</tr>
<tr>
<td>4</td>
<td>The Metabolites: An Integrated metabolite Profiling and Lipidomics Approach For The Detection of The Adulteration of Beef With Pork, Analyst</td>
<td>(Mi et al., 2019)</td>
<td>2019</td>
<td>Raw pork meat</td>
<td>LC-MS</td>
<td>61 glycerolipids, 17 glycerophospholipids, 4 sterol lipids, 2 sphingolipids, 3 polyketides, 7 fatty acyls and 6 prenol lipids, Fatty acid</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>(Trivedi et al., 2016)</td>
<td>2016</td>
<td>Meat</td>
<td>GC-MS and UHPLC-MS</td>
<td></td>
</tr>
</tbody>
</table>

**Note:** NMR (Nuclear Magnetic Resonance); HPLC (High Performance Liquid Chromatography); UHPLC-QTOF-MS (Ultra-High Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry); LC-MS (Liquid Chromatography-Mass Spectrometry); GC-MS (Gas Chromatography-Mass Spectrometry); UHPLC-MS (Ultra-High Performance Liquid Chromatography-Mass Spectrometry).
Table 3. The summary of advantage & disadvantage for metabolomic and lipidomics in the authentication of meat product.

<table>
<thead>
<tr>
<th>No</th>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Metabolomic</td>
<td>Has a high accuracy value, Comprehensive analysis of the entire metabolome associated with the complete complement of small molecule, have been used to analyze organic components</td>
<td>Requires proper instruments for analytical processes such as LC-MS, Metabolomic analysis equipment is expensive.</td>
<td>(Trivedi et al., 2016; Emwas et al., 2019)</td>
</tr>
<tr>
<td>2</td>
<td>Lipidomic</td>
<td>Areas explored in food analysis and more specifically meat adulteration, can be used detection meat with quickly</td>
<td>The data obtained are limited to lipid compounds and sub lipids</td>
<td>(Trivedi et al., 2016)</td>
</tr>
</tbody>
</table>

Note: LC-MS (Liquid Chromatography-Mass Spectrometry)
Fig. 1. Workflow for metabolomics analysis in meat samples
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