JBP
Journal of Biochemicals and Phytomedicine
eISSN: 2958-8561

Identification of Animal Species in Meat Products Using Chemometrics-Based High-Performance Liquid Chromatography: A Systematic Review

Sohrab Pirhadi [1*], Nabi Shariatifar [2], Mohadeseh Pirhadi [2]

[1] Computer Science, Department of Computer Science and Information Technology, Institude of Advanced Studies in Basic Sciences (IASBS), Zanjan, Iran
[2] Food Safety & Hygiene Division, Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article Type:
Review

Article History:
Received: 12 Dec 2023
Revised: 12 Mar 2024
Accepted: 15 Mar 2024
Available online: 30 Jun 2024

Keywords:
Adulteration,
Meat products,
Chemometrics,
Liquid chromatography

*Corresponding author:
E-mail: sohrab.pirhadi94@gmail.com

ABSTRACT

Ensuring accurate meat species identification and animal authentication in meat products is crucial for promoting fair trade and empowering consumers to make informed decisions. Concerns about food fraud have grown due to significant financial and trust-related consequences. Chromatographic techniques combined with chemometrics are widely used in the food industry to confirm the origin and authenticity of food products. Liquid chromatography-mass spectrometry (LC-MS) is highly sensitive and can accurately identify meat metabolites and lipids. This systematic review focuses on analytical methods for the quantification and authentication of meat products. Data were collected from authoritative databases such as Web of Science, Scopus, and PubMed between January 2014 and February 2023. The review examines the use of LC-MS to identify the nature and origin of food. Studies demonstrate the high efficiency of LC-MS due to advanced techniques for extraction, concentration, and identification, enabling the separation and identification of trace amounts with high accuracy and specificity. Identifying peptide markers is crucial for developing these methods. LC-MS/MS stands out for its accuracy in detecting food adulteration, relying on the reliability of proteotypic peptides unique to the animal species used as food. The large amount of data generated during mass spectrometry necessitates the use of chemometric methods to analyze LC-Q-TOF data. The combination of LC-Q-TOF and chemometrics has become a powerful tool for the qualitative and quantitative analysis of food ingredients. The results indicate that LC-MS/MS proteomics tools effectively identify and specify species markers in various food types, including meat, delivering accurate and statistically robust results.

Please cite this paper as:
Pirhadi S, Shariatifar N, Pirhadi M. Identification of Animal Species in Meat Products Using Chemometrics-Based High-Performance Liquid Chromatography: A Systematic Review. Journal of Biochemicals and Phytomedicine. 2024; 3(1): 53-58. DOI: 10.34172/jbp.2024.11.

Introduction

Throughout history, the issue of food consumption has been a primary concern due to its negative impact on food quality and its potentially harmful health consequences, such as diseases and poisoning (Bansal et al., 2017). Meat, as a crucial source of protein and energy, has become a fundamental component of human diets worldwide. However, balancing the growing demand for meat with the limitations of its production poses a significant challenge for many countries (Hogeveen, Steeneveld, and Wolf, 2019; Ahmadi et al. 2021; Negahdari et al., 2021).

In some industrialized countries, per capita meat consumption is high, reaching approximately 21 kg. Conversely, in Iran, per capita consumption is only 11 kg, which is insufficient to meet the body's requirements and may lead to malnutrition (Arabkhaleghi, Mirshokraei, and Seifi, 2022). Producing meat products that do not match the product label is typically considered fraudulent. It is essential to consider consumers' preferences, religious beliefs (e.g., pork being forbidden in Islam), and health concerns (e.g., food allergies) in meat production (Ballin, 2010).

Fraudulent practices in meat production can include various factors, such as the origin of the meat (e.g., gender, age), substitution of meat (using different tissues or animal species than those advertised), and the inclusion of different types of fats and proteins. This can also involve using expired raw materials instead of fresh ones, improper cooking methods, and incorrect quantities of meat in products (Hajimohammadi et al., 2020). Food adulteration, the act of intentionally or unintentionally reducing food quality by adding foreign particles or removing value-added substitutes from the original food item, remains a significant concern (Bansal et al., 2017).

According to Bouzembrak and Marvin (2016), the most common form of food fraud is substituting food with similar and cheaper alternatives that are difficult for consumers and conventional analytical methods to identify. Perpetrators not only reduce food quality but also pose health risks to consumers (Esteki, Shahsavari, and Simal-Gandara, 2019). Food authentication involves analyzing food samples to ensure they meet label specifications, including checking geographic origin, production, processing, and storage conditions. Reliable and efficient analytical methods are crucial for food authentication, enabling health authorities to detect illegal activities and develop better policies and methods to control food production processes.

Techniques such as liquid chromatography (LC), gas chromatography (GC), tandem mass spectrometry (MS), and vibrational spectroscopy (NIR and MIR) are commonly used for food authentication. Additional techniques include Raman spectroscopy, hyperspectral imaging (HSI), nuclear magnetic resonance spectroscopy (NMR), light and infrared microscopy, electronic spin resonance spectroscopy (ESR), polymerase chain reaction (PCR), and enzyme-linked immunosorbent assay (ELISA) (Sarlaki and Aboonajmi 2019). Esteki et al. highlighted the precision and sophisticated analysis required for chromatographic fingerprinting over other techniques (Esteki, Shahsavari, and Simal-Gandara, 2019).

Methods

Search strategy

In this study, we reviewed articles published between 2014 and 2022 with a focus on keywords such as "adulteration," "meat products," "chemometrics," and "liquid chromatography" included in their title. We searched for these articles using the Google Scholar, Scopus, and PubMed databases.

Study assortment

The flowchart of the study design has been indicated in figure 1. Records identified through database searching by a combination of keywords.

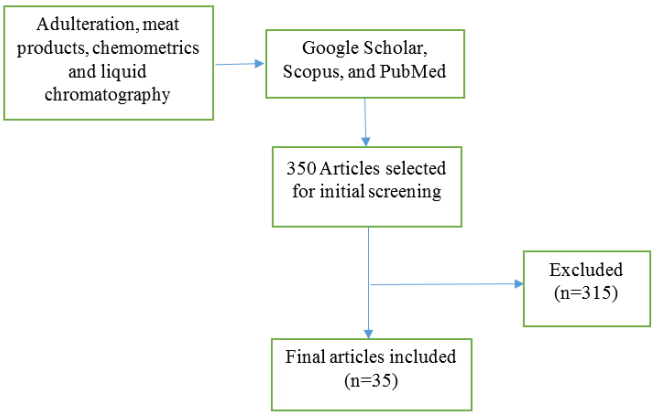

Figure 1. Flowchart of the study

Results and Discussion

Authentication of Meat Products

Authentication of meat products involves the intentional substitution or addition of ingredients that result in a lower nutritional value than the original food. This practice can also include providing false or incorrect information about the food, its ingredients, or its packaging, all aimed at generating greater economic profit (Jurica et al., 2021). Food adulteration is the practice of intentionally or unintentionally adding foreign substances or removing valuable components from food, resulting in decreased quality. Over the past few decades, the demand for meat and meat products has risen significantly, leading to an imbalance between production and consumption stages. Industrial processes can modify the primary raw materials and involve several steps in creating the final product.

Types of Fraud in Meat Products

The primary areas of fraud in the meat industry include the following (Candoğan, Altuntaş, and İğci 2021; Nešić, Stojanović, and Baltić 2017):

1. Origin of Meat and Nutritional Diet of Animals: Misrepresentation of the source and dietary regimen of the animals.
2. Replacing Meat Ingredients: Substitution with tissues, fat, protein, and other non-meat ingredients.
3. Manipulating Processing Methods: Including cooking at higher than permissible temperatures to reduce preparation time.
4. Adding Non-Meat Ingredients: Incorporation of substances such as water or additives, including melamine.

Many studies have examined meat adulteration; some are discussed in Table 1.

Table 1. Studies by infrared spectroscopy for detecting food adulteration

Meat type	Purpose of analysis	Analytical method	Ref
Meat	Hydrophilic interactions in ovine meat's color stability analyzed by HILIC-MS-based metabolomics	HPLC	(Subbaraj et al., 2016)
Pig, cattle, sheep, deer, chicken, and duck	Identification of seven animal species in meat products by LC-MS/MS	LC-MS/MS	(Zhang et al., 2022)
Beef, pork, chicken and duck	Find distinguishing markers for four main types of meat by LC-MS/MS	LC-MS/MS	(Kim et al., 2017)
Duck, goose and chicken	LC-MS methods for monitoring three poultry species in processed meat products using species-specific peptide-based feasibility	LC–MS	(Fornal and Montowska, 2019)
Beef burgers	Detecting particular non-meat proteins and peptides present in beef burgers	LC-Q-TOF-MS/MS	(Mikołajczak, Fornal, and Montowska, 2018)
Chicken, Rabbit, duck, Sheep, horse, Deer, buffalo	way to measure the amount of beef and pork used in Bolognese sauce	UHPLC	(Prandi et al., 2017)
Pork	The method of HPLC-MS/MS was optimized to detect markers of pork protein in meat products.	HPLC	(Nalazek-Rudnicka et al., 2019)
Chicken, pork, Sheep, duck, Goose	LC-Q-TOF-MS/MS identification of allergenic protein peptide markers in poultry products containing soy, milk, and egg whites	LC-Q-TOF-MS/MS	(Montowska and Fornal, 2018)
Chicken, Pork, sheep, Duck, beef	Searching for heat-stable peptide biomarkers in cooked meats from five different animal species	LC-Q-TOF-MS/MS	(Wang et al., 2018)
Chicken, Pork, Horse, beef	Discovering novel peptides to identify horse meat in heavily processed foods	LC-Q-TOF-MS/MS	(Claydon et al., 2015)
Horses and donkeys	A new peptide marker using the enzyme chymotrypsin can differentiate horses from donkeys with reliability in ZooMS	LC-MALDI-TOF-MS/MS	(Paladugu et al. 2023)
Beef, pork, horse, and chicken	Rapid detection of peptide markers for meat authentication using surface analysis mass spectrometry with ambient liquid extraction in both raw and cooked meat	LESA-MS/MS	(Montowska et al., 2014)
Pork, beef and chicken	Development of potential non-solvent pork peptide markers among solvent beef and chicken using chemometrics	LC-MS	(Yuswan et al., 2018)
Rabbit, chicken and pork	An evaluation of rabbit-specific peptide markers was conducted using LC-QTOF-MS for the purpose of meat quantification	LC-QTOF-MS	(Stachniuk et al., 2014)
Chicken, duck, goose, guinea fowl, ostrich, pheasant, pigeon, and quail	The method used to identify different poultry species involves high-performance liquid chromatography-tandem mass spectrometry for nine types	HPLC-MS/MS	(Häfner, Kalkhof, and Jira, 2021)
Pork	Detection of pork adulteration in the meat of Pangasius hypoptalmus using liquid chromatography-mass spectrometry	LC-HRMS	(Windarsih, Warmiko, et al., 2022)
Pork and beef meatballs	Using LC-HRMS based chemometric, pork detection in beef meatballs can be identified	LC-HRMS	(Windarsih, Riswanto, et al., 2022)
Pork, beef	Investigation of an integrated metabolite and lipidomics method to detect adulteration of beef with pork	UHPLC-MS	(Trivedi et al., 2016)
Pork	Characterization and discrimination of selected Chinese domestic pork using an LC-MS-based lipidomic approach	LC-MS	(Mi et al., 2019)
Lamb	Authentication of lamb meat by liquid chromatography time-of-flight mass spectrometry	UHPLC-QTOF	(Wang et al., 2021)

Fornal and Montowska suggested that LC-MS methods can authenticate food, monitor compliance with label claims, and detect adulteration in poultry-containing food products (Fornal and Montowska, 2019). Mikołajczak et al. reported that it is possible to identify and verify specific non-meat proteins and peptides in beef hamburgers using the LC-Q-TOF-MS/MS device (Mikołajczak, Fornal, and Montowska, 2018). Parandi et al. demonstrated that the quantity of beef and pork in Bolognese sauce can be determined using a UHPLC device (Prandi et al., 2017). Nalazek-Rudnicka et al. detected pork protein markers in meat products using HPLC-MS/MS (Nalazek-Rudnicka et al., 2019). Wang et al. indicated that eighteen heat-stable peptide biomarkers were found in cooked meats of five animal species using the LC-Q-TOF-MS/MS method (Wang et al., 2018). Claydon et al. discovered thermostable horse-specific peptides capable of detecting low levels of horsemeat in mixed species (Claydon et al. 2015). Montowska et al. found that LESA-MS is specific for peptide digest analysis and is simpler and faster than other meat speciation methods (Montowska et al., 2014).

Liquid Chromatography and Chemometrics in Food Authentication

Nowadays, liquid chromatography methods are highly efficient due to the use of advanced extraction, concentration, and identification techniques, enabling the separation and accurate identification of trace amounts with high specificity (Zarean Baniasadi et al., 2019). Liquid chromatography (LC), when combined with various mass spectrometry (MS) detectors, has led to significant advancements and new applications in proteomics. The food industry increasingly uses LC-MS methods to identify protein-derived peptides resistant to heat treatment, which are essential for food authentication purposes. The accuracy of identifying food adulteration depends on the reliability of proteotypic peptides unique to the animal species used as food, making peptide marker identification a critical step in developing new LC-MS/MS methods. Various types of meat have been subjected to LC-MS/MS proteomics tools, successfully identifying and characterizing species markers (Kotecka-Majchrzak et al., 2021). Table 1 presents some studies conducted in this field.

Yuswan et al. reported consistent results for identifying peptide markers using LC-MS methods and chemometric analysis (Yuswan et al., 2018). Windarsih et al. concluded that combining non-targeted metabolomics with LC-HRMS and chemometrics is a promising standard method for authenticating solvents in processed meat (Windarsih, Riswanto, et al., 2022).

Conclusions

Food has always been a crucial and sensitive factor in human life. Throughout history, wars and migrations have often been driven by the need to access areas with more abundant food supplies. Unfortunately, recent years have seen an increase in the adulteration of animal-derived food products. To prevent food fraud, product characteristics must be well-defined and based on evidence. Food fraud scandals can erode consumer confidence and cause significant economic and public health damage. There are various scientific methods available to ensure food safety and prevent fraud in the supply chain. This review article discusses several liquid chromatography techniques utilized for this purpose. A metabolomic approach that employs LC-MS/MS and chemometrics can effectively authenticate meat product solvents, providing a robust means to combat food fraud.

Declarations

Conflict of interest

The authors declare no conflict of interest.

Acknowledgement

Authors would like to thank from School of Public Health, Tehran University of Medical Sciences and Health Services, Tehran, Iran.

Consent for publications

The authors approved the manuscript for publication.

Funding/support

None.

Authors' contributions

SP, NS, and MP had the same contribution in writing, editing and approving the manuscript.

Ethical considerations

All ethical issues, including plagiarism, misconduct, data fabrication, falsification, double publication, or submission redundancy, have been fully considered.

References

Ahmadi M, Alikord M, Pirhadi M, Masoumi S, Molaee Aghaee E. Screening and investigation of microbial and chemical properties of meat products in Hamadan Province, Iran during 2012-2015. Infection Epidemiology and Microbiology. 2021; 7: 327-35.

Arabkhaleghi F, Mirshokraei P, Seifi H. Search for Bovine Herpes Virus I in Iranian frozen semen. Journal of Veterinary Research. 2022; 77. DOI: 10.22059/jvr.2022.345709.3284.

Ballin NZ. Authentication of meat and meat products. Meat Science. 2010;86:577-87.

Bansal S, Singh A, Mangal M, Mangal AK, Kumar S. Food adulteration: Sources, health risks, and detection methods. Critical Reviews in Food Science and Nutrition. 2017; 57: 1174-89. DOI: 10.1080/10408398.2014.967834.

Bouzembrak Y, Marvin HJP. Prediction of food fraud type using data from Rapid Alert System for Food and Feed (RASFF) and Bayesian network modelling. Food Control. 2016;6 1: 180-7. DOI: 10.1016/j.foodcont.2015.09.026.

Candoğan K, Gunes Altuntas E, İğci N. Authentication and quality assessment of meat products by Fourier-transform infrared (FTIR) spectroscopy. Food Engineering Reviews. 2021; 13: 66-91. DOI: 10.1007/s12393-020-09251-y.

Claydon AJ, Grundy HH, Charlton AJ, Romero MR. Identification of novel peptides for horse meat speciation in highly processed foodstuffs. Food Additives & Contaminants: Part A. 2015; 32: 1718-29. DOI: 10.1080/19440049.2015.1075256.

Esteki M, Shahsavari Z, Simal-Gandara J. Food identification by high performance liquid chromatography fingerprinting and mathematical processing. Food Research International. 2019;122:303-17. DOI: 10.1016/j.foodres.2019.04.025.

Fornal E, Montowska M. Species-specific peptide-based liquid chromatography–mass spectrometry monitoring of three poultry species in processed meat products. Food Chemistry. 2019;283:489-98.

Häfner L, Kalkhof S, Jira W. Authentication of nine poultry species using high-performance liquid chromatography–tandem mass spectrometry. Food Control. 2021;122:107803. DOI: 10.1016/j.foodcont.2020.107803.

Hajimohammadi B, Fattahi K, Kavyani Yekta Z, Sadeghinezhad J, Morovvati H, Akhondzadeh Basti A. Experimental study of the histological method for quantitative detection of meat in Kabab and cooked sausage model. Journal of Veterinary Research. 2020;75. DOI: 10.22059/jvr.2020.239180.2681.

Hogeveen H, Steeneveld W, Wolf CA. Production diseases reduce the efficiency of dairy production: A review of the results, methods, and approaches regarding the economics of mastitis. Annual Review of Resource Economics. 2019;11:289-312. DOI: 10.1146/annurev-resource-100518-093954.

Jurica K, Brčić Karačonji I, Lasić D, Bursać Kovačević D, Putnik P. Unauthorized food manipulation as a criminal offense: Food authenticity, legal frameworks, analytical tools and cases. Foods. 2021;10:2570. DOI: 10.3390/foods10112570.

Kim GD, Seo JK, Yum HW, Jeong JY, Yang HS. Protein markers for discrimination of meat species in raw beef, pork and poultry and their mixtures. Food Chemistry. 2017; 217: 163-70. DOI: 10.1016/j.foodchem.2016.08.100.

Kotecka-Majchrzak K, Kasałka-Czarna N, Sumara A, Fornal E, Montowska M. Multispecies identification of oilseed-and meat-specific proteins and heat-stable peptide markers in food products. Molecules. 2021; 26: 1577. DOI: 10.3390/molecules26061577.

Mi S, Shang K, Li X, Zhang CH, Liu JQ, Huang DQ. Characterization and discrimination of selected China's domestic pork using an LC-MS-based lipidomics approach. Food Control. 2019; 100: 305-14. DOI: 10.1016/j.foodcont.2019.02.001.

Mikołajczak B, Fornal E, Montowska M. LC–Q–TOF–MS/MS identification of specific non-meat proteins and peptides in beef burgers. Molecules. 2018; 24: 18. DOI: 10.3390/molecules24010018.

Montowska M, Alexander MR, Tucker GA, Barrett DA. Rapid detection of peptide markers for authentication purposes in raw and cooked meat using ambient liquid extraction surface analysis mass spectrometry. Analytical Chemistry. 2014; 86: 10257-65. DOI: 10.1021/ac502449w.

Montowska M, Fornal E. Detection of peptide markers of soy, milk and egg white allergenic proteins in poultry products by LC-Q-TOF-MS/MS. LWT. 2018;87:310-17. DOI: 10.1016/j.lwt.2017.08.091.

Nalazek-Rudnicka K, Kłosowska-Chomiczewska I, Wasik A, Macierzanka A. MRM–MS of marker peptides and their abundance as a tool for authentication of meat species and meat cuts in single-cut meat products. Food Chemistry. 2019;283:367-74. DOI: 10.1016/j.foodchem.2019.01.007.

Negahdari S, Sabaghan M, Pirhadi M, Alikord M, Sadighara P, Darvishi M, Nazer M. Potential harmful effects of heavy metals as a toxic and carcinogenic agent in marine food-an overview. Egyptian Journal of Veterinary Sciences. 2021;52:379-85. DOI: 10.21608/ejvs.2021.83716.1245.

Nešić K, Stojanović D, Baltić ŽM. Authentication of meat and meat products vs. detection of animal species in feed–what is the difference? In: IOP Conference Series: Earth and Environmental Science. 2017. DOI: 10.1088/1755-1315/85/1/012043.

Paladugu R, Richter KK, Valente MJ, Gabriel S, Detry C, Warinner C, Barrocas Dias C. Your horse is a donkey! Identifying domesticated equids from Western Iberia using collagen fingerprinting. Journal of Archaeological Science. 2023; 149: 105696. DOI: 10.1016/j.jas.2022.105696.

Prandi B, Lambertini F, Faccini A, Suman M, Leporati A, Tedeschi T, Sforza S. Mass spectrometry quantification of beef and pork meat in highly processed food: Application on Bolognese sauce. Food Control. 2017; 74: 61-9. DOI: 10.1016/j.foodcont.2016.11.032.

Sarlaki E, Aboonajmi M. Authentication and identification of food adulterants based on fingerprinting techniques and chemometric tools. Food Hygiene. 2019; 9: 89-112. DOI: 10.30495/JFH.2019.669289.

Stachniuk A, Kozub A, Czeczko R, Montowska M, Fornal E. LC-QTOF-MS evaluation of rabbit-specific peptide markers for meat quantitation. Journal of Food and Drug Analysis. 2014; 30: 223. DOI: 10.38212/2224-6614.3403.

Subbaraj AK, Brad Kim YH, Fraser K, Farouk MM. A hydrophilic interaction liquid chromatography–mass spectrometry (HILIC–MS) based metabolomics study on colour stability of ovine meat. Meat Science. 2016; 117: 163-72. DOI: 10.1016/j.meatsci.2016.02.028.

Trivedi DK, Hollywood KA, Rattray NJW, Ward H, Trivedi DK, Greenwood J, et al. Meat, the metabolites: An integrated metabolite profiling and lipidomics approach for the detection of the adulteration of beef with pork. Analyst. 2016; 141: 2155-64. DOI: 10.1039/C6AN00108D.

Wang GJ, Zhou GY, Ren HW, Xu Y, Yang Y, Guo LH, Liu N. Peptide biomarkers identified by LC–MS in processed meats of five animal species. Journal of Food Composition and Analysis. 2018; 73: 47-54. DOI: 10.1016/j.jfca.2018.07.004.

Wang J, Xu Z, Zhang H, Wang Y, Liu X, Wang Q, Xue J, Zhao Y, Yang S. Meat differentiation between pasture-fed and concentrate-fed sheep/goats by liquid chromatography quadrupole time-of-flight mass spectrometry combined with metabolomic and lipidomic profiling. Meat Science. 2021; 173: 108374. DOI: 10.1016/j.meatsci.2020.108374.

Windarsih A, Riswanto FDO, Abu Bakar NK, Yuliana ND, Dachriyanus, Rohman A. Detection of pork in beef meatballs using LC-HRMS based untargeted metabolomics and chemometrics for halal authentication. Molecules. 2022;27:8325. DOI: 10.3390/molecules27238325.

Windarsih A, Warmiko HD, Indrianingsih AW, Rohman A, Ulumuddin YI. Untargeted metabolomics and proteomics approach using liquid chromatography-Orbitrap high resolution mass spectrometry to detect pork adulteration in Pangasius hypopthalmus meat. Food Chemistry. 2022;386:132856. DOI: 10.1016/j.foodchem.2022.132856.

Yuswan MH, Aizat WM, Lokman AA, Desa MNM, Mustafa S, Junoh NM, Yusof ZNB, Mohamed R, Mohmad Z, Lamasudin DU. Chemometrics-assisted shotgun proteomics for establishment of potential peptide markers of non-halal pork (Sus scrofa) among halal beef and chicken. Food Analytical Methods. 2018;11:3505-15. DOI: 10.1007/s12161-018-1327-6.

Zarean Baniasadi F, Ahmadi M, Rokni N, Golestan L, Shahidi SA. Evaluation of four common antibiotic classes in the muscle and liver of chickens slaughtered in Tehran by LC-MS/MS. Veterinary Research & Biological Products. 2019; 32: 55-63. DOI: 10.22092/VJ.2019.125604.1565.

Zhang M, Li Y, Zhang Y, Kang C, Zhao W, Ren N, Guo W, Wang S. Rapid LC-MS/MS method for the detection of seven animal species in meat products. Food Chemistry. 2022; 371: 131075. DOI: 10.1016/j.foodchem.2021.131075.