Tutor/s

Dr. Oscar Núñez Burcio
Departament d'Enginyeria Química i Química Analítica

Grau de Química

Treball Final de Grau

Characterization and authentication of meat using HPLC-UV fingerprinting and chemometrics.
Caracterització i autenticació de carn mitjançant empremtes HPLC-UV i quimiometria.

Alexandra Santomá Martí

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"…I vaig inclinar la meva atenció cap a l'estudi, vaig ocupar llargues hores a devorar els principis de la ciència fins adquirir els coneixements que ignorava. I ara, Pepeta meva, quan ja em trobo a la tardor de la meva vida no puc més que donar les gràcies a Déu per el que vaig tenir la possibilitat d'aprendre..."

Joaquim Martí i Soler.

Barcelona, Novembre 1927

Aquest treball no hauria estat possible sense el suport i la guia de moltes persones. En primer lloc, vull agrair especialment al meu tutor, el Dr. Oscar Núñez, per la seva implicació, ajuda i consells al llarg d'aquests mesos fos el dia que fos, però, sobretot, per les oportunitats que m'ha brindat i tots els coneixements i passió per la recerca que m'ha transmès, sempre estaré molt agraïda. Al Marc per la seva paciència, el seu suport diari i per ser una font constant d'ànims quan més ho necessitava, encara que ell es trobés en la mateixa situació. Als meus pares, l'Ana i el Ricky per estar allà sempre i animar-me a seguir endavant no només durant aquests mesos, sinó tota la meva vida. I, com no, a les meves germanes, la Tala i la Oli que encara que no s'ho creguin són les persones que més m'animen i m'alegren en el meu dia a dia. A tota la meva família, en especial als meus avis, la Pitita, l'avi Ricardo i la Wity per estar sempre allà i interessar-se tant per com anava el meu treball. A la Titi per transmetre'm des del dia 1 la seva passió per la química, i la Marta per ser una font incondicional d'energia positiva. I a tots els meus tiets i cosins, la Ali, el Alex, el Gonza, el Dito, la Elena, la Jimena, el Bosco, la Martina, la Nora i l'Erik que d'una manera o altre han estat al meu costat. A les meves amigues i a tots els companys de FAAST pel suport i els moments viscuts aquests mesos. I com no, mencionar al meu avi Joaquim, que tot i que mai podrà arribar a llegir-se aquest treball, sé que és el que més orgullós hauria estat d'ell. I el primer que m'hauria dit hauria sigut: "Esto és un 10 subrayado".

REPORT

IDENTIFICATION AND REFLECTION ON THE SUSTAINABLE DEVELOPMENT GOALS (SDG)

Food fraud is a significant issue that raises concerns related to health, ethics, and religious practices. It threatens consumer trust and highlights the need for reliable authentication methods. While many laborious techniques have been developed, this project focuses on evaluating a simpler and more sustainable approach to classify and authenticate meat, contributing to the detection and prevention of meat fraud.

The research carried out in this Final Degree Project aligns with several Sustainable Development Goals (SDG), demonstrating its potential contribution to sustainable development. The project has a significant impact on the 5 Ps: Planet, People, Prosperity, Peace, and Partnership.

Regarding the Planet, this project promotes the authentication of meat products through non-targeted metabolomic approaches, contributing to the reduction of food waste caused by mislabelling or fraud. By ensuring the accuracy of food labelling, it supports the development of sustainable food systems and reduces the environmental impact of the food industry. This aligns with Goal 12 (Responsible Consumption and Production), particularly target 12.3, which aims to reduce food waste, and target 12.6, which encourages companies to adopt sustainable practices.

In terms of People, this study plays a crucial role in protecting consumers from the economic and health risks associated with food fraud. It enhances public trust in food systems by ensuring access to safe and high-quality meat products, thus improving consumer confidence and well-being. This aligns with Goal 3 (Good Health and Well-being), specifically target 3.9, which seeks to reduce illnesses caused by food contamination. Moreover, by guaranteeing access to safe and nutritious food, this project supports Goal 2 (Zero Hunger), target 2.1, which focuses on food security.

From a Prosperity perspective, the methodology developed supports fair market practices by helping local producers maintain their competitive edge. Ensuring the integrity of labels such as organic or PGI certifications allows small-scale farmers and businesses to thrive in a competitive market, promoting economic growth and sustainability. This aligns with Goal 8 (Decent Work and Economic Growth), target 8.4, which promotes resource-efficient production and economic growth without harming the environment.

The project also contributes to Peace by enhancing transparency and accountability within the food industry. Reducing fraudulent practices decreases tensions and conflicts caused by unfair competition, fostering a more equitable and trustworthy food supply chain. Additionally, collaboration between universities, industry, and regulatory bodies enhances transparency in food authentication, supporting Goal 17 (Partnerships for the Goals), which emphasizes the importance of partnerships for achieving the SDGs. Such collaborations can drive further technological advances in food safety and authentication.

Finally, if expanded, this project could result in a greater social impact by establishing a global database for meat authentication. Using advanced mass spectrometry, specific biomarkers for authenticity could be identified, further supporting food safety and contributing to a more sustainable and equitable food system. This aligns with Goal 9 (Industry, Innovation and Infrastructure), specifically target 9.5, which promotes scientific research and technological innovation for sustainable development.

CONTENTS

1. SUMMARY

In this work, a simple and cost-effective high-performance liquid chromatography with ultraviolet detection (HPLC-UV) fingerprinting methodology was developed and applied after a water-based extraction procedure to obtain chemical descriptors for meat authentication through chemometrics. Meat samples from different species (lamb, beef, pork, rabbit, quail, chicken, turkey, and duck) as well as different non-genetic attributes (protected geographical indications, organic production, and Halal and Kosher meats) were analysed. The resulting HPLC-UV fingerprints (segment from minute 7 to 17) were subjected to principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) for classification and authentication purposes.

The PLS-DA models demonstrated excellent classification performance, achieving sensitivity and specificity values higher than 100% and 99.3% for calibration and cross-validation, respectively, with classification errors below 0.4% when meat species were analysed. Furthermore, a hierarchical classification decision tree based on consecutive dual PLS-DA models achieved 100% accuracy in the prediction of 48 unknown meat samples. Multiclass PLS-DA models addressing geographical origin, organic production, and Halal and Kosher attributes also achieved satisfactory results, with sensitivity and specificity values exceeding 91.2% and classification errors below 6.9%.

Additionally, fraudulent meat adulteration cases involving PGI, organic, and Halal or Kosher meats were evaluated using partial least squares (PLS) regression, allowing the detection and quantification of adulteration levels between 15% and 85%, with prediction errors below 6.6%.

This study demonstrates the suitability of the proposed HPLC-UV fingerprinting methodology for addressing meat authenticity issues beyond the capabilities of genetic methods. It provides a reliable, sustainable, and accessible tool for improving food safety and transparency in the meat industry.

Keywords: Meat authentication, HPLC-UV fingerprinting, Chemometrics, Food Fraud, Meat Geographical Origin, Meat Production Practices, Meat Adulteration, Partial Least Squares-Discriminant Analysis, Principal Component Analysis, Partial Least Squares Regression.

2. RESUM

En aquest treball s'ha desenvolupat i aplicat una metodologia senzilla i econòmica de cromatografia líquida d'alta eficàcia amb detecció ultraviolada (HPLC-UV) per obtenir empremtes metabolòmiques no dirigides com a descriptors químics per a l'autenticació de carn mitjançant tècniques quimiomètriques. Es van analitzar mostres de carn de diferents espècies (xai, vedella, porc, conill, guatlla, pollastre, gall dindi i ànec) així com diferents atributs no genètics (indicacions geogràfiques protegides, producció ecològica i carns Halal i Kosher). Les empremtes HPLC-UV obtingudes (segment del minut 7 al 17) es van sotmetre a anàlisi de components principals (PCA) i anàlisi discriminant de mínims quadrats parcials (PLS-DA) per a la classificació i autenticació.

Els models PLS-DA van demostrar una excel·lent capacitat de classificació, assolint valors de sensibilitat i especificitat superiors al 100% i 99,3% per a la calibració i la validació creuada, respectivament, amb errors de classificació inferiors al 0,4% quan es van analitzar les espècies de carn. A més, un arbre de classificació jeràrquic basat en models consecutius de PLS-DA duals va aconseguir una precisió del 100% en la predicció de 48 mostres desconegudes. Els models PLS-DA multiclasse per a l'origen geogràfic, la producció ecològica i els atributs Halal i Kosher també van mostrar resultats satisfactoris, amb valors de sensibilitat i especificitat superiors al 91,2% i errors de classificació inferiors al 6,9%.

A més, es van avaluar casos d'adulteració fraudulenta de carn que involucraven carns amb IGP, ecològiques i Halal o Kosher mitjançant regressió de mínims quadrats parcials (PLS), permetent la detecció i quantificació de nivells d'adulteració entre el 15% i el 85%, amb errors de predicció inferiors al 6,6%.

Aquest estudi demostra l'eficàcia de la metodologia d'empremtes HPLC-UV proposada per abordar problemes d'autenticitat de carn més enllà de les capacitats dels mètodes genètics. Proporciona una eina fiable, sostenible i accessible per millorar la seguretat alimentària i la transparència en la indústria càrnia.

Paraules clau: Autenticació de carn, Empremtes HPLC-UV, Quimiometria, Frau alimentari, Origen geogràfic de la carn, Pràctiques de producció de carn, Adulteració de carn, Anàlisi discriminant de mínims quadrats parcials, Anàlisi de components principals, Regressió de mínims quadrats parcials.

3. INTRODUCTION

3.1. FOOD AUTHENTICATION AND FOOD FRAUD

The quality and transparency in food is an increasingly concern among society, which is attracting significant attention. Today the prices people pay for products that claim to possess a set of attributes, like sustainably produced, organic, or from a certain place, which any has been proven to be of higher nutritional quality, are even excessive in some cases. And this situation has led to a growth in fraudulent practices. Food fraud can be defined as the deliberate modification, marketing, misrepresentation or adulteration of food products for profit that endangers consumers not only economically, but it can also affect their health and well-being. These practices include incorrect labelling (like declaring something is organic when it's not), replacing high-quality ingredients with inferior ones, or even adding undeclared substances to copy the appearance or properties of other products. Because of the danger involved on these practices, food fraud is prohibited throughout the world and its rapid detection should be guaranteed. So here is where food authentication takes place.

Food authentication is the analytical process that verifies that a food is in compliance with its label description [1]. This may include verifying aspects such as its origin involving the species or protected geographical indication or, the method of production such as whether it is organic, conventional or if it follows a religious food practice, among others. In other words, food authentication is the process by which it is verified that a food is not fraudulent. And this is vital as it offers protection to consumers from fraudulent acts [2].

In Europe, origin is one of the main authenticity matters relating to food. The European Union has provided legal specific names with regulations for foods with a specific quality, mainly through PDO (Protected Designation of Origin), PGI (Protected Geographical Indication), and TSG (Traditional Specialities Guaranteed) labels [1]. New regulations also consider terms like "mountain product" and "product of island farming" (1151/2012 EU Regulation) [3] to recognize unique qualities associated with some areas. Although these names give confidence to consumers and support rural farmers, it is not free from being defenceless against fraud.

Returning to this, it is important to mention that every year, due to the interests of society for food with different attributes that may seem of higher nutritional quality and with new advances in food technology and the complexity of the food-chain, fraud cases are growing continuously more. Besides, some categories of food are especially susceptible to fraud. Olive oil, honey, wine, meat and dairy products are typical targets of fraudulent practices for illicit economic benefits. For example, olive oil can be mixed with less expensive vegetable oils; honey can be sweetened with sugar syrups. Meat in particular can be replaced with lower-quality or different species of meat that would not be detected by inspection alone and would be mislabelled.

3.2. MEAT AUTHENTICATION AND MEAT FRAUD

Meat and meat products are among the most consumed food items globally, contributing in a significant way to human diets due to their high-quality protein content and rich nutrient profile. Meat represents approximately 40% of the total global protein consumption. This reality, combined with population growth, leads to a rising demand for these types of products [4]. However, it is not only about the protein, beyond this, meat is a high source of essential micronutrients such as iron, zinc, and selenium, as well as vitamins A, B6 and B12 [5].

Apart from the nutritional benefits that meat offers, consumers in recent years have shown growing interest in attributes such as the species, origin, and production methods of the meat, among others [6]. These attributes that appeal to consumers also make it susceptible to fraudulent practices. This highly valued characteristics lead to incidents such as species substitution, mislabelling, and adulteration. For instance, the substitution of high-value meats with cheaper alternatives has been documented, and recent cases include the sale of conventionally raised chicken labelled as organic in Australia in 2024 and the detection of undeclared chicken DNA in packaged sliced turkey in the United Kingdom in 2022 [7].

In addition to quality concerns, cultural and religious practices add complexity to meat authentication. For example, Halal and Kosher certifications require not only specific animal species but also adherence to particular slaughtering methods. Similarly happens with products with a protected geographical indication (PGI) label, as they depend on the verification of their regional origin and their traditional production methods, which are also an easy target to fraud.

Regarding fraud detection, there are different methods to determine adulteration. The identification of animal species can be easily determined using DNA techniques such as Polymerase Chain Reaction which is highly effective for detecting species substitution in meat [8], among other techniques [9]. However, several meat authentication issues not involving different meat species cannot be solved by means of genetic detection tools. These cases are linked to cultural and religious practices, such as those in Halal or Kosher products. Other examples include the geographical origin of meat products. Additionally, aspects like sustainable meat production, organic farming, or animal welfare are cases where fraudulent activities will not either be detected by genetic methods. To overcome these limitations, phenotypic approaches, including metabolomics and chemometrics [10], are emerging as robust solutions to ensure the authenticity and integrity of meat products.

In conclusion, although the nutritional qualities of meat make it a key component of diets around the world, they also make it highly susceptible to fraud. Addressing these issues demands a comprehensive approach that integrates traditional genetic methods with advanced analytical techniques, guaranteeing the authenticity, quality, and safety of meat products in today's constantly evolving global market.

3.2.1. Organic Meat

Organic meat is different from conventionally produced meat because of the way animals are raised, fed and other organic standards set by national governments and international organizations that must be followed to comply with the term "organic food" [11]. This type of meat comes from animals that are raised in accordance with strict guidelines to ensure animal welfare, protect the environment, enhance biodiversity, and provide safe food.

In organic meat production, the methods used do not involve the use of synthetic pesticides, chemical fertilizers or genetically modified organisms. The use of growth hormones, chemical food additives, or techniques such as irradiation are also not allowed [11]. For example, in the European Union (EU), some of the rules require that animals are fed natural feed, have access to outdoor areas, and live in conditions that respect their well-being [12]. This means the animals have more space to move and are not given any chemicals in their diet.

In the other hand, organic farming also has a positive influence on biodiversity as it uses local breeds of animals that are better adapted to the environment [13].

Consumers are increasingly choosing organic meat because they associate it with healthier and safer meat. Organic meat is often seen as free from harmful substances like pesticides, antibiotics, or artificial additives, and this makes these products attractive to those wanting to reduce their exposure to these chemicals. In addition, it is associated with higher concentrations of beneficial compounds, such as antioxidants, which may have better nutritional attributes [13].

However, previous studies concluded that there is not enough scientific evidence to ensure that organic products are inherently safer, healthier, or more nutritious than conventionally produced ones. This may be because organic farming systems focus more on sustainable production practices rather than directly targeting the final product's quality or safety. Despite this, the ethical and environmental benefits of organic farming still make it a preferred choice for many consumers [14].

Nonetheless, the rules for organic meat are not uniform globally, and variations exist between regions and countries. For example, in the EU, Council Regulation 2092/91 and Council Regulation 1804/99 [15,16] provide a detailed framework for organic meat production, outlining specific rules for prophylaxis, therapy, and slaughtering age, among others. In the United States, the Organic Foods Production Act (OFPA) of 1990 serves as the point of reference for organic certification [12]. Despite these differences, the main goal is the same: to produce high-quality meat that is more natural and sustainable.

In conclusion, organic meat production is characterized by its commitment to ecological and ethical principles, differentiating it from conventional practices through its emphasis on sustainability, animal welfare, and food safety. These attributes make organic meat an appealing choice for health-conscious and environmentally aware consumers.

3.2.2. Protected Geographical Indication Meat

Protected Geographical Indication (PGI) meat is a distinct category within the meat industry, which is distinguished from conventional meat through its geographical origin and traditional production methods used. This distinction appears from the European Union's quality assurance schemes, which aim to recognize and promote products of superior quality due to their unique characteristics derived from their regions of production [17]. According to the European Commission, “a geographical indication is a distinctive sign used to identify a product as originating in the territory of a particular country, region or locality where its quality, reputation or other characteristic is linked to its geographical origin” [18].

For a product to obtain the PGI distinction, it must follow several strict criteria. These requirements include a detailed description of the product and production process, proof of origin within clearly defined geographical boundaries, and an indication of the link between its quality or characteristics and its geographical origin [19]. Moreover, the product must be produced, transformed, or processed within the designated area, and compliance with these standards is strictly controlled by the authorities of each EU member state [19].

In Spain, the significance of geographical origin is particularly noticeable in the context of PGI meat products. As of 2022, Spain had registered twenty raw meat products under the PGI scheme, including beef, pork, lamb, goatling, ox, and chicken [20]. These products are distinguished by the regions where the animals are raised, which play a crucial role in imparting unique qualities to the meat.

In the case of lamb, the Aragón territory is of particular interest for its climatic conditions which have favoured the development of a sheep subsector. Several sheep breeds in Aragón possess unique traits that set them apart from other sheep commonly slaughtered in Spain. The traditional lamb breeding system in this region involves a grazing phase, typically conducted in mountainous areas, followed by a stabling phase [21]. This combination of practices gives the meat its particular taste and quality, making it an excellent example of the influence of geographical and cultural factors on PGI products.

3.2.3. Halal and Kosher Meat

The global demand for Halal and Kosher meat products has significantly increased in recent years. This is not only due to the religious practices of Muslim and Jewish communities but also because these products are often associated with higher quality and meticulous preparation standards. While these dietary practices have their roots in religious doctrines, they have gained popularity among non-religious consumers who perceive them as healthier and more reliable options. Despite their differences, both Halal and Kosher dietary laws emphasize cleanliness, purity, and strict adherence to specific guidelines to follow about food preparation and diet in the holy books of both Islam and Judaism, making their certifications highly looked for. Consequently, customers are ready to pay higher prices for Halal and Kosher foods because of its special requirements in manufacturing and supply chain which have made the products susceptible to adulteration [22].

Halal, an Arabic term meaning “permitted, allowed lawful or licit”, refers to any food allowed to consume by a Muslim as it complies with Islamic law, as defined in the holy book Quran. Any food which has the potential to or causes a bad effect on the mind, body or spirit is Haram: "unauthorized or illicit” [23].

Halal meat must come from animals that are considered lawful according to Shariah law, such as cows, goats, ducks, chickens, turkeys, and camels, among others, and they must have been slaughtered according to Islamic Rituals. Prohibited animals include pork, meat of animals that are shot or who died of disease and were not slaughtered and animals slaughtered in a name other than that of Allah.

Meat that is slaughtered according to the code of Islam is called “zabiha”, or lawful. The slaughtering process requires the animal to be alive and healthy at the time of slaughter. The animal is given full access to feed and water, then a mature, pious Muslim must

perform the act manually using a sharp, stainless-steel knife to sever the respiratory tract, oesophagus, and jugular vein in a single motion while reciting the phrase “In the name of Allah.” The blood is drained completely, as consuming blood is strictly prohibited. The entire process underscores the importance of hygiene, humane treatment of animals, and spiritual significance, ensuring that the meat remains clean and lawful for consumption [23].

Kosher, a term derived from Biblical Hebrew meaning “fit”, refers to food prepared according to Jewish dietary laws known as Kashrut. Food unfit to eat is referred to as non-Kosher or "treif," which means unclean.

For meat to be considered Kosher, it must come from ruminant animals with split hooves, such as cows, sheep, and goats. Pigs, for example, despite having split hooves, are not Kosher because they do not chew their cud. Similarly, poultry such as chickens, ducks, turkeys and gees lack front toes and are not ruminant, these are considered Kosher depending on how they are slaughtered. The slaughter, or “Shechita”, must be carried out by a religious butcher known as “shochet”, who is specifically trained. The animal is rendered unconscious as quickly as possible ensuring it remains unaware of pain as it is being slaughtered. It also must be properly restrained. This is particularly important in Halal and Kosher practices, where the animal's neck must be positioned correctly and kept relatively still during the process. Proper restraint minimizes the animal's stress and prevents pain, bruising, or injury [24]. The trachea, oesophagus, carotid arteries, and jugular veins are severed in one swift and virtually instantaneous stroke, using a sharp, unblemished knife to make a swift and precise incision that minimizes the animal’s suffering. After the slaughter, the animal is submitted to an inspection to ensure it is free of any diseases or defects, as these would render the meat non-Kosher. Unlike Halal practices, Kosher laws prohibit the consumption of the sciatic nerve and fat surrounding the organs, making further processing necessary for full compliance [23].

The global market for Halal and Kosher meat products is rapidly expanding due to changing consumer preferences. Halal foods, once primarily consumed by Muslims, are now popular among non-Muslims for their perceived cleanliness, health benefits, and safety. Similarly, Kosher food has gained traction, especially in the United States, where most consumers are non-Jewish, and Europe [22]. This trend is driven by the high standards of quality, healthfulness, and food safety associated with these products, as confirmed by a Mintel survey highlighting these factors as key reasons for purchasing Kosher food [25].

In conclusion, Halal and Kosher dietary laws represent more than religious obligations; they represent principles of hygiene, ethics, and spirituality. And even though many consumers view these certifications as guarantees of ethical treatment of animals, rigorous inspection standards, and high-quality products, the increasing demand also poses challenges, such as the risk of adulteration and fraudulent labelling. Ensuring the authenticity and sanctity of Halal and Kosher products requires strict oversight throughout the supply chain, from the slaughterhouse to the store display [22].

3.3. Analytical Approaches for Meat Authentication

Genetic methods, such as polymerase chain reaction (PCR), are widely used in the detection of meat adulteration involving different animal species. These DNA-based techniques have proven to be highly specific and sensitive, making them the preferred choice for identifying the genetic origin of meat in complex food matrices. For example, to detect the presence of rat meat which is considered haram (illicit) by Muslims in Halal meat, PCR techniques have been successfully employed [26]. PCR amplification relies on the binding of specific oligonucleotides to a target DNA sequence, enabling the production of millions of copies flanked by these primers. The most basic PCR method used to identify non-halal meat involves amplifying DNA fragments and then verifying their size through agarose gel electrophoresis [26]. This approach enables precise identification of species, helping to ensure compliance with religious dietary laws such as Halal and Kosher. However, despite their efficacy in species identification, these genetic tools are unable to provide information about phenotypic attributes of the meat. Characteristics such as organic production, geographical origin, or slaughtering practices, which are important for product labelling and consumer trust, cannot be determined by means of genetic tools. To address these limitations, metabolomic analytical methodologies are emerging as the best options to address these authentication issues.

The metabolome is the complete set of small molecules, known as metabolites, which include metabolic intermediates, hormones, signalling molecules, and secondary metabolites. These compounds can be found in any biological sample, including food derived

from animals or plants. These metabolites not only reflect the genetic composition of the animal but also the phenotypic information which includes the external factors that have affected the animal throughout its life [27]. Any environmental factor, such as the geographical origin, feeding practices like organic production, animal welfare, or the method of slaughter like Halal or Kosher methods, influences the animal's metabolome and, therefore, the metabolites present in its meat or derived products. For this reason, metabolomic studies are highly valuable for verifying meat authenticity and addressing fraudulent practices that cannot be detected only through genetic methods.

Two main analytical strategies are employed in metabolomic studies: targeted (profiling) and non-targeted (fingerprinting) approaches. Targeted metabolomics are focused on the specific determination of a given group of metabolites or families of metabolites using well-defined methodologies, offering high precision, selectivity, and reliability [28]. This approach has benefits like low detection limits, quantitative analysis and simpler data interpretation and analysis, but is limited by the need for purified standards and prior knowledge of the compounds of interest.

In contrast, non-targeted metabolomics aims to capture all detectable metabolites in a sample without prior knowledge of their identity [28]. This comprehensive strategy is also impartial in identifying highly abundant molecules, offers a high throughput and may allow, if necessary, the discovery of new compounds. However, interpreting the resulting data can be challenging and usually requires combination with advanced techniques of chemometric analysis to reduce the comprehensive data sets into smaller collections of controllable variables, particularly for tasks like classification and authentication.

Liquid chromatography (LC) combined with high-resolution mass spectrometry (HRMS) is commonly used in both targeted and non-targeted metabolomic studies and are among the most powerful techniques to address meat authentication issues by metabolomics [29]. For example, LC-HRMS untargeted metabolomics and chemometrics has been recently proposed for the detection of pork in beef meatballs for halal authentication studies [30]. These techniques enable the separation and identification of a wide range of metabolites with high accuracy and resolution. Despite the significant advantages of HRMS, such as its high-resolution and accurate mass measurements, its high cost and the requirement for specialized personnel limit its accessibility, especially in control laboratories and developing countries. To address these challenges, more affordable and straightforward non-targeted methods, such as spectroscopic techniques (UV-vis, near-infrared, or Fourier transform infrared, among other) and liquid chromatography with spectroscopic detection, are gaining popularity in food analysis, including meat products.

In this study, high-performance liquid chromatography with ultraviolet detection (HPLC-UV) was used as a cost-effective alternative for metabolomic fingerprinting. This technique records a wide range of instrumental chemical responses from samples without requiring the identification of individual compounds. The resulting fingerprints serve as chemical descriptors, which are then analysed using chemometric methods to discriminate/classify and authenticate meat products. Chemometric tools, such as principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA), facilitate the interpretation of complex datasets and enhance the robustness of authentication methodologies. The integration of chemometrics with HPLC-UV fingerprinting offers an effective way to address meat authenticity issues.

3.4. Chemometric Techniques in Food Analysis

Chemometrics is a scientific discipline that integrates principles from chemistry, mathematics, and statistics to analyse complex chemical datasets. It has become an essential tool in modern food analysis, allowing researchers to process and interpret large amounts of data derived from techniques such as high-performance liquid chromatography (HPLC), mass spectrometry (MS), and nuclear magnetic resonance (NMR) [31]. These techniques generate multivariate data, and chemometrics helps to uncover patterns and relationships that would be impossible to detect otherwise. Through the application of mathematical and statistical models, chemometrics establishes a systematic approach for the authentication and characterization of food products.

The term "chemometrics" refers to the application of mathematical and statistical methods to extract meaningful information from chemical data. In food analysis, this discipline plays a crucial role in handling large datasets and interpreting results effectively. Chemometric techniques are typically divided into two main categories: pattern recognition and multivariate calibration. Pattern recognition is used to identify similarities and differences between samples and can be further divided into unsupervised and

supervised methods [32]. Unsupervised pattern recognition like Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) are exploratory tools that do not require prior knowledge of sample groupings. Supervised methods, on the other hand, such as Partial Least Squares-Discriminant Analysis (PLS-DA), use labelled data to build models for classification and discrimination [32]. It's important to highlight that the grouping patterns observed in PCA can indicate the robustness and reliability of the PLS-DA models developed using the same data [31].

On the other hand, multivariate calibration, the second main category, is used for quantitative analysis to predict the concentration of target analytes using multivariate data [31]. Common techniques in this category include Multiple Linear Regression (MLR) and Partial Least Squares (PLS) regression. These methods are widely applied in food analysis to provide reliable and accurate results.

Chemometrics plays an important role in food analysis for several reasons. It helps in identifying specific biomarkers that distinguish different types of food, detecting adulterants, and verifying product authenticity [31]. For instance, in the analysis of meat products, chemometric techniques have been used to identify non-halal meats and specific metabolites associated with pork [31]. Additionally, chemometric models need to be validated to ensure their reliability and predictive accuracy. Methods like cross-validation and permutation tests are commonly used for this purpose, providing confidence in the models developed [31].

The following subsections introduce some of the key chemometric methods used in food analysis. These methods PCA, PLS-DA and PLS regression, are all essential tools for analysing complex chemical data and ensuring the quality and authenticity of food products.

3.4.1. Principal Component Analysis

PCA is a widely used chemometric method that simplifies and reduces the dimensionality of complex datasets [33]. It decomposes the original data matrix, referred to as the X-matrix, into uncorrelated and orthogonal variables known as principal components (PCs). These PCs retain most of the variance present in the original data, with the first principal component (PC1) accounting for the highest variance, followed by PC2, which captures the second highest, and so on. PCA helps to identify trends, outliers, and groupings within the data by representing the PCs as axes. The number of PCs required to describe the dataset depends on the level of correlation among the variables, as higher correlations result in fewer PCs, thereby reducing the model's dimensionality. In food analysis, PCA is particularly useful for distinguishing features such as species, origin, or other properties of food products. By analysing score plots of the PCs, patterns can be observed, and samples with similar properties cluster together, allowing researchers to draw conclusions about the relationships between different samples. PCA is also often used as a preprocessing step prior to the application of supervised methods like PLS-DA [32]. This preprocessing helps to make the data more manageable and enhances the efficiency of the supervised models.

3.4.2. Partial Least Squares-Discriminant Analysis

PLS-DA is a widely used supervised classification method in chemometrics. It is particularly effective for distinguishing between predefined classes in complex datasets [32]. PLS-DA models the relationship between the X-matrix, containing the independent variables, and the Y-matrix, which represents the class labels. This correlation is expressed through latent variables (LVs), which summarize the data structure and are visualized in score plots. These plots help to display the distribution of samples and their grouping according to class similarities. PLS-DA seeks to maximize the covariance between the X and Y matrices, creating a predictive model that highlights the differences between sample classes.

3.4.3. Partial Least Squares Regression

PLS regression is a highly chosen chemometric method for developing calibration models, especially when dealing with large and complex datasets. PLS simultaneously processes the information from both the predictor variables (X-matrix) and the predicted variables (Y-matrix), identifying a set of latent factors that explain the maximum variance in both matrices while maximizing their correlation [33]. This dual focus on predictors and predicted variables distinguishes PLS from traditional calibration methods, which often prioritize one over the other. The algorithm’s ability to balance these two aspects makes it particularly effective in applications

such as food analysis, where it is crucial to uncover hidden relationships and build reliable predictive models. PLS is widely recognized for its speed and efficiency, enabling researchers to analyse large datasets rapidly while maintaining high accuracy in the results.

4. OBJECTIVES

This project aims to evaluate the potential of HPLC-UV fingerprinting combined with chemometric tools for the characterization and authentication of meat samples, focusing on detecting adulteration and differentiating meat based on species, geographical origin, and production systems. To achieve this objective, the following steps will be carried out:

1. The methodology used in previous studies based on using the entire HPLC-UV fingerprint will be refined by focusing on specific chromatographic segments aiming to enhance the resolution and specificity of the analysis.
2. A reproducible and robust method for obtaining HPLC-UV fingerprints from meat samples will be developed and optimized.
3. Principal component analysis will be applied to evaluate the reproducibility and robustness of the HPLC-UV fingerprints and to explore patterns within the dataset.
4. Partial least squares-discriminant analysis will be used to classify meat samples according to species, geographical origin, and production systems, evaluating the discriminant capability of the method.
5. The discriminant effectiveness of the proposed methodology for detecting adulteration in meat samples will be evaluated, particularly in distinguishing differences within the same species but different production systems (e.g., organic, Halal, and Kosher).
6. The performance of the proposed method will be validated through cross-validation and the prediction of unknown samples.

5. EXPERIMENTAL SECTION

5.1. CHEMICALS

Acetonitrile (Supergradient ACS quality for UHPLC) was obtained from Panreac AppliChem (Barcelona, Spain). Formic acid (≥96%) was purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Water (Milli-Q quality) was purified using a Milli-Q Reference A+ system from Merck (Darmstadt, Germany), and filtered through 0.22 µm nylon filters.

5.2. INSTRUMENTATION

An HPLC instrument (Agilent 1100 Series, Waldbronn, Germany) equipped with a binary pump (G1312A model), an automatic sample injector (WPALS G1367A model), a diode-array UV-visible detector (G1315B model), and a PC with the Agilent Chemstation for LC software was employed. Chromatographic separation for meat fingerprinting was performed using a Phenomenex Kinetex® C18 (100 × 4.6 mm I.D., 2.6 µm partially porous particle size) column (Torrance, California, USA). The separation was performed under a universal gradient elution program, with 0.1% formic acid in water as solvent A and acetonitrile as solvent B as the mobile phase components. A flow rate of 0.4 mL/min was employed.

The chromatographic separation was based on a previously developed method by N. Aijon [34]. Briefly, the gradient elution program used was: 3% solvent B from 0 to 1 minute (initial conditions), followed by a linear gradient increase to 95% solvent B over 1 to 20 minutes. This was maintained at 95% solvent B from 20 to 22 minutes, then returned to 3% solvent B in 0.1 minutes, with these initial conditions held until minute 25 to allow column re-equilibration. UV-visible spectra were recorded in the range of 190-400 nm,

with HPLC-UV fingerprints at 280 nm selected as chemical descriptors for meat authentication. Aqueous meat extracts were injected using a 5 µL injection volume.

5.3. SAMPLES

5.3.1. Meat Samples

Meat samples from eight different animal species were obtained from various local markets and supermarkets in Spain. These species included Rabbit, Beef, Pork, Lamb, Chicken, Turkey, Quail, and Duck, with 20 samples collected for each type, resulting in a total of 160 samples analysed. The samples were examined to assess the ability of the HPLC-UV fingerprinting method to distinguish between different animal species.

To evaluate the authenticity of meat based on its geographical origin, lamb samples from two distinct regions in Spain were analysed. These included 20 samples of lamb produced in Catalonia and 20 samples with a Protected Geographical Indication (PGI) label from Aragon.

For assessing the authenticity of different production systems, chicken and beef samples were used. Both non-organic and organic chicken meat samples were obtained, all produced in Catalonia, with 20 samples for each category. Similarly, beef samples included both non-organic and organic types, the organic ones sourced from two different producers in Catalonia, with 20 samples per category.

Additionally, to investigate production practices related to religious communities, lamb and beef samples were analysed. For lamb, 20 Halal and 20 non-Halal samples were used. For beef, the analysis included 20 samples each of standard beef, Halal beef, and Kosher beef.

5.3.2. Sample Treatment

Sample treatment was based on a previously developed method by N. Aijon [34]. Briefly, the initial preparation of the meat samples involved the elimination of the fatty parts, followed by grinding the meat using a meat processor. Approximately 1 g of the ground meat was then weighed into 50 mL PTFE centrifuge tubes (Serviquimia, Barcelona, Spain) and mixed with 10 mL of water. The mixture was vigorously agitated for 1 minute using a VibraMix Vortex (OVAN, Barcelona, Spain). Subsequently, ultrasound-assisted extraction (UAE) was performed for 15 minutes in a 5510 Branson ultrasonic bath (Barcelona, Spain). After the extraction process, the samples were centrifuged for 5 minutes at 4000 rpm using a Rotina 420 centrifuge (Hettich, Tuttlingen, Germany). The resulting aqueous extracts were filtered into 2 mL HPLC vials using 0.45 µm nylon syringe filters (FILTER-LAB, Barcelona, Spain) and stored at 4°C until further analysis.

5.4. DATA ANALYSIS

5.4.1. Meat Classification Studies

The aqueous meat extracts were analysed randomly using the proposed HPLC-UV method to minimize potential variations caused by instrumental drift. To ensure repeatability and robustness, a quality control (QC) sample was prepared by mixing 50 µL of each extract, and this QC was injected after every 10 samples. The chromatographic data were exported to an Excel spreadsheet using Unichrom software (New Analytical Systems, Minsk, Belarus), resulting in HPLC-UV fingerprints composed of 3750 absorbance signal values at 280 nm for each sample, as a function of chromatographic retention time. For further analysis, only the retention time segment between 7 and 17 minutes was used, reducing each fingerprint to 1500 absorbance value variables.

Chemometric analysis was performed by constructing data matrices from the non-targeted HPLC-UV fingerprints. PCA was applied as an exploratory tool to assess the distribution of the meat samples and monitor QC sample behaviour. PLS-DA was employed as a supervised classification method to distinguish samples based on meat species, geographical origin, or production system (e.g., organic vs. non-organic, Halal vs. non-Halal, Kosher vs. non-Kosher). The analyses were conducted using SOLO 8.6 chemometrics software (Eigenvector Research, Manson, Washington, USA). For PCA, the X-data matrix contained the absorbance values recorded at 280 nm within the 7-17 minutes chromatographic segment for each sample. This same X-data matrix was used for PLS-DA,

excluding the QC samples, alongside a Y-data matrix that defined the sample classes (species, origin, or production system). The optimal number of latent variables (LVs) for PLS-DA models was determined by identifying the first relevant minimum in the cross-validation (CV) error using the Venetian blind method. When necessary, ellipses were manually drawn on score plots to visually delineate sample clusters.

The performance of the PLS-DA models was evaluated in terms of sensitivity, specificity, and accuracy. Sensitivity measures the model's ability to correctly identify positive samples, calculated as TP/(TP + FN), where TP (true positives) refers to the number of samples that truly belong to the studied class and are correctly classified by the model, and FN (false negatives) refers to the number of samples that belong to the studied class but are incorrectly classified as not belonging to it. Specificity assesses the model's capability to correctly identify negative samples, calculated as TN/(TN + FP), where TN (true negatives) represents the number of samples that do not belong to the studied class and are correctly identified as such, and FP (false positives) represents the number of samples that do not belong to the studied class but are incorrectly classified as belonging to it. Accuracy provides a general measure of the classification performance, calculated as (TP + TN)/TS, where TS is the total number of samples. For example, in the classification of samples as belonging to the class "chicken" vs. "not chicken," TP corresponds to chicken samples correctly classified as chicken, FN represents chicken samples incorrectly classified as not chicken, TN refers to non-chicken samples correctly classified as not chicken, and FP corresponds to non-chicken samples incorrectly classified as chicken.

In addition to standard PLS-DA models, in the study based on the classification of meat species, a hierarchical classification decision tree was implemented to enhance the overall classification performance. This decision tree consisted of a series of consecutive paired PLS-DA models, constructed using the Hierarchical Model Builder (HMB) approach. Initially, 30% of the samples from each class were randomly selected to form a prediction set, comprising 48 meat samples for the meat species authentication study, while the remaining 70% (112 samples) were used to develop the calibration models.

In the first step, a multiclass PLS-DA model was applied to the 112 calibration samples to identify the sample class with the highest degree of discrimination. Once this class (referred to as "class 1") was identified, a paired PLS-DA model was constructed to separate class 1 from the other sample groups, forming the first rule node of the classification tree. This model was then used to classify the 48 prediction samples. Then, samples from class 1 that were correctly assigned, as well as samples from other classes that were incorrectly assigned to class 1, were removed from the prediction set. However, samples from class 1 that were misclassified as belonging to another group remained in the prediction set for further analysis. In the second step, the remaining calibration samples (excluding those from class 1) were used to develop a new multiclass PLS-DA model to identify the next most discriminated class, referred to as "class 2." A paired PLS-DA model was then constructed to differentiate class 2 from the remaining sample groups, forming the second rule node of the decision tree. This process of selecting the most discriminated class, constructing paired PLS-DA models, and refining the prediction set was repeated iteratively until all classification tree rule nodes were defined and each sample class was successfully discriminated. The performance of the hierarchical decision tree was also evaluated based on sensitivity, specificity, and accuracy metrics, applied to the calibration, cross-validation, and prediction stages, ensuring robust and reliable classification results.

5.4.2. Detection and Quantitation of Meat Adulteration

The potential of the developed HPLC-UV fingerprinting methodology to identify and quantify meat adulteration was evaluated using partial least squares (PLS) regression. Six adulteration situations were studied, involving mixtures of meats from different geographical origins or production systems, including comparisons between organic and non-organic meats, Halal and non-Halal meats, and Kosher and non-Kosher meats. For this purpose, PLS calibration models were constructed using adulteration levels of 0% (pure meat), 20%, 40%, 60%, 80%, and 100% (pure adulterant meat). Validation of the models was performed using adulteration levels of 15%, 25%, 50%, 75%, and 85%. Each adulteration level, both for calibration and validation, was prepared in quintuplicate to ensure the reliability of the results.

Additionally, a 50% adulteration level was included as a quality control (QC) extract to assess the reproducibility and robustness of the method. The PLS X-data matrix was constructed using the HPLC-UV fingerprints recorded at 280 nm over the chromatographic

segment from 7 to 17 minutes for each blended meat sample and QC. The Y-data matrix, on the other hand, corresponded to the adulteration percentages. The optimal number of latent variables (LVs) for the PLS models was determined through cross-validation using the Venetian blinds method.

6. RESULTS AND DISCUSSION

6.1. CLASSIFICATION OF MEAT SAMPLES BASED ON MEAT SPECIES

6.1.1. HPLC-UV Fingerprints of Meat Samples and Segment Optimization

This study continues the work carried out in a previous Final Degree Dissertation within the research group, which focused on the classification and authentication of meat using chromatographic metabolomic fingerprints [34]. Meat samples from eight different animal species: beef, pork, lamb, chicken, rabbit, turkey, quail, and duck were analysed. 1 g of each meat sample was extracted with water following the extraction procedure described in section 5.3.2. Chromatographic fingerprints of the aqueous meat extracts were obtained by reversed-phase separation using a porous-shell C18 column and 0.1% aqueous formic acid and acetonitrile as mobile phase components under universal gradient elution conditions (section 5.2). All the samples were analysed randomly and injecting a QC solution every 10 samples.

The initial project commented before focused on the analysis of the entire fingerprint obtained from the chromatographic profiles of the different animal species. However, in the present study, a specific segment of the fingerprint, corresponding to the retention time range of 7 to 17 minutes, was selected to investigate whether this targeted approach could improve the classification and differentiation between species. Figure 1 illustrates a representative example of a full chromatogram alongside the selected segment between 7 and 17 minutes, which was identified as containing the most characteristic peaks for distinguishing between species. This visual comparison emphasizes the reasoning behind selecting this specific interval to enhance the accuracy of species classification.

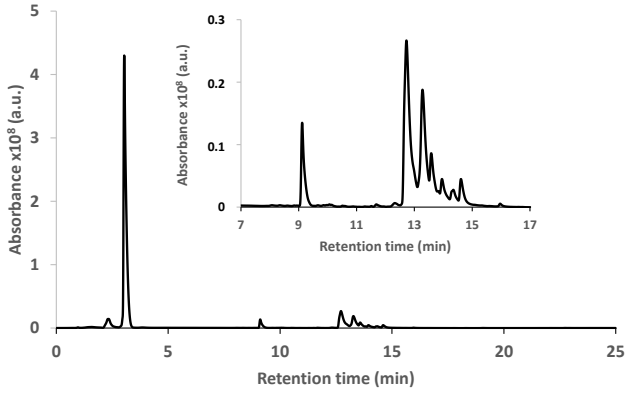

Figure 1. Non-targeted HPLC-UV chromatogram (280 nm) of a pork sample extracted with pure Milli-Q water.

As can be seen in Figure 1, focusing on the full fingerprint, there are some polar compounds eluting close to the column dead volume, and then other groups of compounds that are selecting within the time range from 7 to 17 minutes. In general, all the samples showed the same signal at the beginning of the chromatograms, thus, in the present study, these signals were not considered for the sample classification studies.

Figure 2 shows the HPLC-UV fingerprints of the 7 to 17 minutes range for each of the eight meat species under study. As can be seen, these chromatograms reveal clear differences in both the number and intensity of peaks, depending on the species. Specifically,

the regions from 9 to 10 minutes and from 12.5 to 15 minutes exhibit the most intense peaks, likely corresponding to key metabolites characteristic of each species. Additionally, other compounds with lower peak signals between 10 and 12.5 minutes may also contribute to the differentiation of species, serving as valuable discriminant descriptors when addressing sample classification.

These results allow us to consider that focusing on the 7 to 17 minutes segment will provide a clear advantage by emphasizing the most relevant chemical information while maintaining the reproducibility of the fingerprints within each species. This targeted approach aligns with the goal of enhancing classification accuracy using optimized fingerprinting data. The following sections will detail the results of subsequent chemometric analyses, including PCA and PLS-DA, to evaluate the effectiveness of this approach in meat species classification.

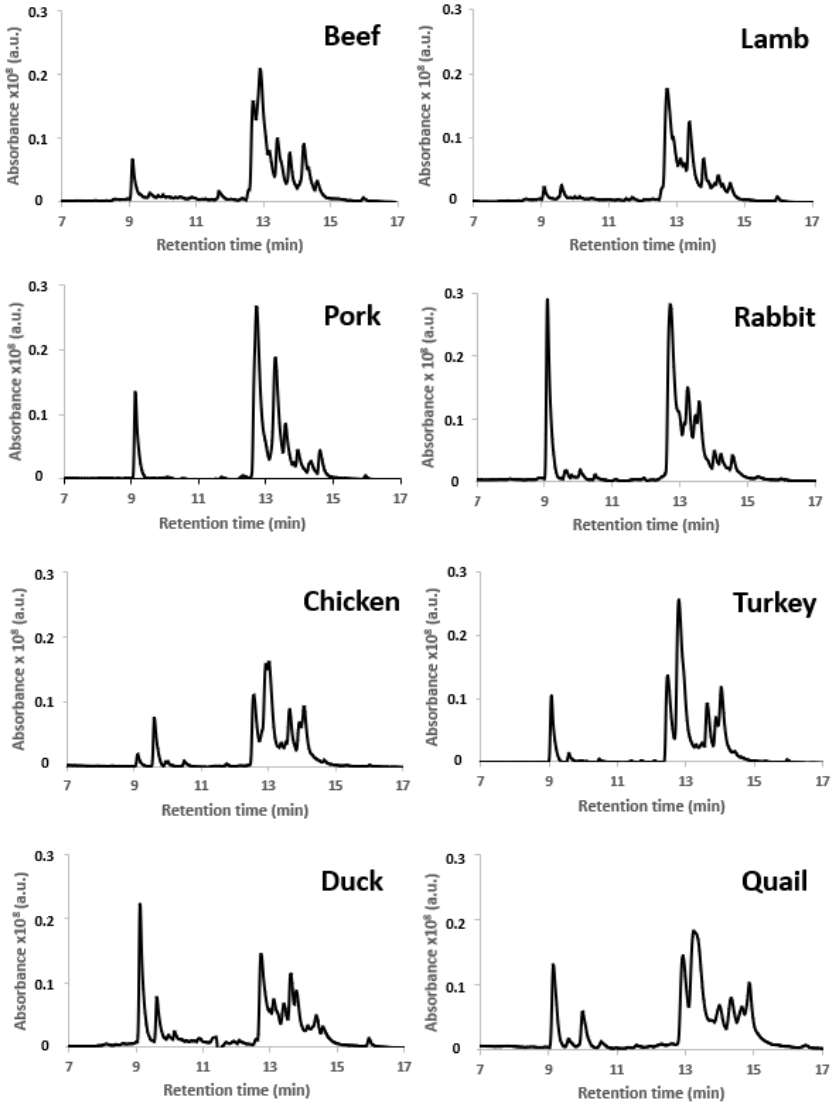

Figure 2. HPLC-UV fingerprints (at 280 nm, segment from 7 to 17 min) for one selected sample of each meat specie under study.

6.1.2. Exploratory non-supervised Principal Component Analysis

After obtaining the HPLC-UV fingerprints for 160 meat samples and the quality controls (QCs), a chemometric approach was applied to evaluate the classification and characterization of the meat samples. The first step involved an exploratory non-supervised PCA. This method was employed to assess the reproducibility and reliability of the experimental procedure by analysing the behaviour of the QCs throughout the sequence, as well as to identify any initial patterns or grouping trends among the different meat species under study.

For this purpose, an X-data matrix was constructed, incorporating the HPLC-UV fingerprints (280 nm, 7 to 17 min segment) of all meat samples and QCs. The matrix dimensions were (180 × 1500), representing Samples + QCs × absorbance values across retention times. The PCA model used 9 principal components (PCs), which accounted for 89.60% of the total variance in the dataset.

Figure 3 displays the PCA score plot for PC1 vs. PC2, showing that QCs cluster near the centre of the plot. This clustering confirms the robustness and reproducibility of the HPLC-UV fingerprinting methodology, as no significant instrumental drifts were observed during the analysis. Additionally, some degree of clustering by meat species is evident, although certain overlaps are present when visualizing only two PCs. For instance, beef and quail samples are well-separated from other species, both showing positive PC1 values but differing along PC2, with quail having positive and beef negative PC2 values. Chicken and turkey samples overlap in the top-left region (negative PC1, positive PC2 values). The remaining species are primarily located in the lower section of the plot (negative PC2 values), with some overlapping and a certain discrimination through PC1.

It is important to note that while the 2D plot of PC1 and PC2 provides a preliminary visualization of sample grouping, complete discrimination between species requires consideration of all 9 PCs included in the PCA model.

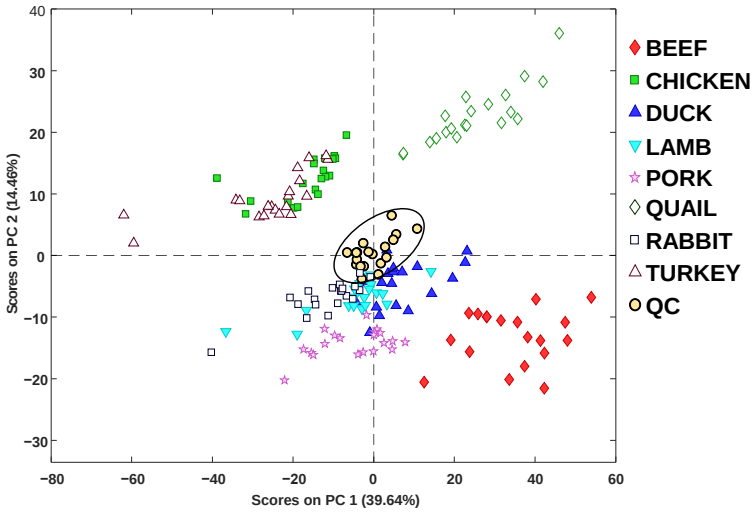

Figure 3. Principal component analysis (PCA) score-plot of PC1 *vs.* PC2 when employing HPLC-UV fingerprints (280 nm, segment from 7 to 17 min) as sample chemical descriptors of the analysed meat and QC samples. The PCA model was built by employing 9 PCs.

6.1.3. Meat Classification based on Animal Species by Partial Least Squares-Discriminant Analysis

Following the verification of the HPLC-UV method's robustness and reproducibility through PCA, a supervised PLS-DA study was conducted to classify the analysed meat samples according to their animal species. In this study, QCs were excluded to focus solely on the classification of the meat samples. An X-data matrix, with dimensions of (160 × 1500), being defined by Samples × absorbance values (as a function of retention time) was employed, while the Y-data matrix defined the sample categories (animal species).

The resulting PLS-DA model employed 7 latent variables (LVs), which explained 83.59% of the total variance in the data. The score plots for LV1 vs. LV2 and LV1 vs. LV2 vs. LV3 are shown in Figure 2. These plots demonstrate a good discrimination capacity, as samples tend to cluster according to their respective species. When visualizing the first three LVs, most species are clearly separated, except for chicken and turkey samples, which overlap slightly. However, full discrimination is achieved when all 7 LVs in the model are considered.

To evaluate the classification performance of the PLS-DA model, sensitivity, specificity, and accuracy values were calculated. Table 1 summarizes the cross-validated prediction results for each meat species. The results show excellent performance, with overall sensitivities, specificities, and accuracies of 100% during calibration, except for a classification error of 0.36% (99.64% accuracy) for quail samples. In cross-validation, most metrics remained at 100%, with minor exceptions: chicken samples had a specificity of 99.3% and a classification error of 0.36%, while quail samples showed sensitivity and specificity values of 99.3% and an accuracy of 99.64% (classification error of 0.36%).

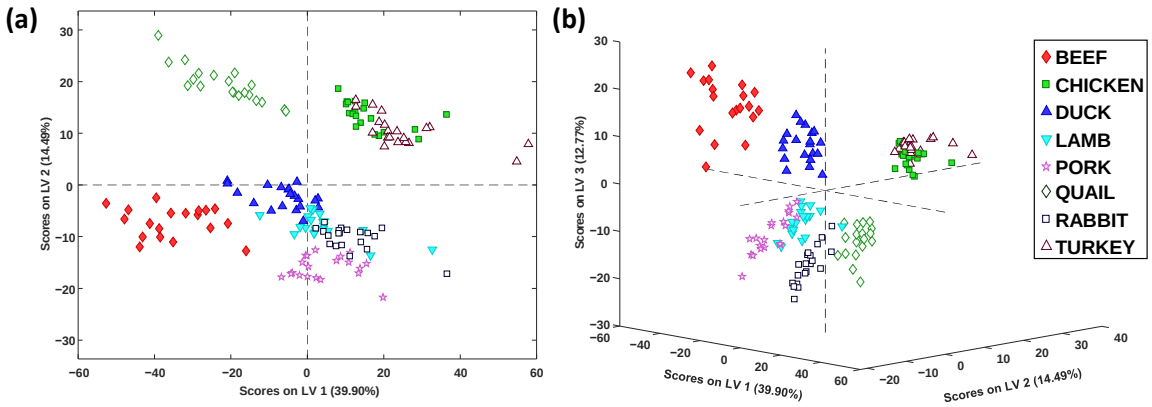

Figure 4. Partial least squares-discriminant analysis (PLS-DA) score plots of (a) LV1 *vs*. LV2 and (b) LV1 *vs*. LV2 *vs*. LV3 when employing HPLC-UV fingerprints (280 nm, segment from 10 to 17 min) as sample chemical descriptors of the analysed meat samples. The PLS-DA model was built by employing 7 LVs.

Table 1. PLS-DA multiclass predictions by cross-validation for the set of meat samples based on animal species.

Sample class variety	Sensitivity (%)		Specificity (%)		Accuracy (classification error, %)	
	Calibration	Cross-validation	Calibration	Cross-validation	Calibration	Cross-validation
Beef	100	100	100	100	0	0
Chicken	100	100	100	99.3	0	0.36
Duck	100	100	100	100	0	0
Lamb	100	100	100	100	0	0
Pork	100	100	100	100	0	0
Quail	100	99.3	100	99.3	0.36	0.36
Rabbit	100	100	100	100	0	0
Turkey	100	100	100	100	0	0

To further explore the classification capabilities of the proposed methodology, PLS-DA models were independently evaluated for mammal and bird meat species. In both cases, an X-data matrix of dimensions (80 × 1500) being defined by Samples × absorbance values (as a function of retention time) were employed, while the Y-data matrix defined the sample categories (animal species). Figure 5 shows the 3D score plots of LV1 vs. LV2 vs. LV3 for (a) mammal meat and (b) bird meat species. In addition, Tables 2 and 3 summarizes the sensitivity, specificity and accuracy values for both mammal and bird meat species, respectively.

As shown in Figure 5a and Table 2, the classification performance for mammal species was excellent, achieving sensitivity, specificity, and accuracy values of 100% across all sample groups. Similarly, for bird species (Figure 5b), the results summarized in Table 3 also demonstrate a high classification performance. However, slight deviations were observed for chicken and turkey samples. Chicken showed a calibration specificity of 95.0% and a cross-validation classification error of 2.5%. Turkey displayed sensitivity and specificity values of 98.3% during cross-validation, with both calibration and cross-validation classification errors of 0.8%. Despite these minor deviations, the overall performance of the PLS-DA models for both mammal and bird species can be considered very acceptable, confirming the suitability of the HPLC-UV fingerprinting approach for meat classification.

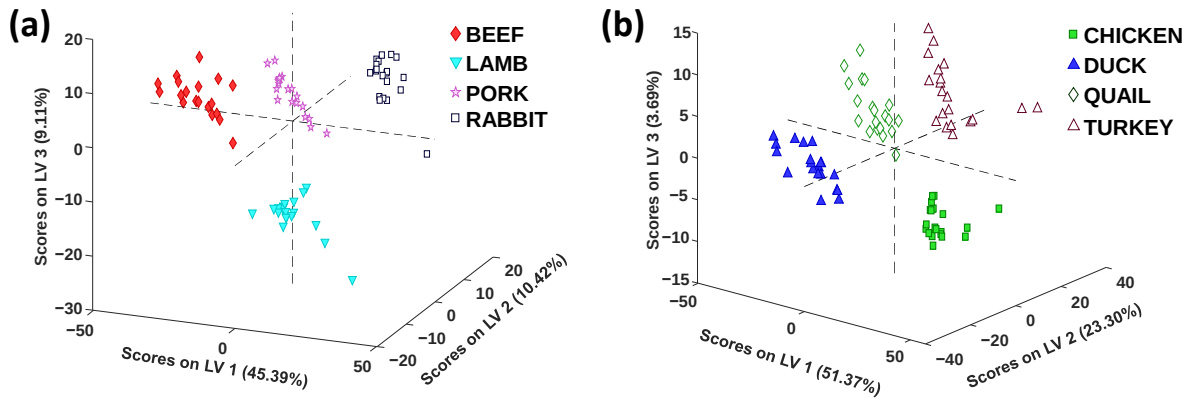

Figure 5. PLS-DA score-plot of LV1 *vs.* LV2 *vs.* LV3 when employing HPLC-UV fingerprints (280 nm, segment from 7 to 17 min) as sample chemical descriptors for the classification of meat samples of (a) mammal species and (b) bird species. Both PLS-DA models were built by employing 3 LVs.

Table 2. PLS-DA multiclass predictions by cross-validation for the set of mammal meat samples based on animal species.

Sample class variety	Sensitivity (%)		Specificity (%)		Accuracy (classification error, %)	
	Calibration	Cross-validation	Calibration	Cross-validation	Calibration	Cross-validation
Beef	100	100	100	100	0	0
Lamb	100	100	100	100	0	0
Pork	100	100	100	100	0	0
Rabbit	100	100	100	100	0	0

Table 3. PLS-DA multiclass predictions by cross-validation for the set of bird meat samples based on animal species.

Sample class variety	Sensitivity (%)		Specificity (%)		Accuracy (classification error, %)	
	Calibration	Cross-validation	Calibration	Cross-validation	Calibration	Cross-validation
Chicken	100	100	95.0	100	0	2.5
Duck	100	100	100	100	0	0
Quail	100	100	100	100	0	0
Turkey	100	98.3	100	98.3	0.8	0.8

To evaluate the potential improvements achieved with the proposed methodology when selecting only the fingerprint segment from minutes 7 to 17 as chemical descriptor, a comparison was made with the results obtained in the previous study conducted within the research group, which analysed the full chromatographic fingerprint (0 to 25 minutes) for meat classification using PLS-DA [34]. Figure 6 shows the PLS-DA score plots of LV1 vs. LV2 for both approaches. While both methods demonstrated good discrimination capabilities, notable differences were observed in the discrimination between species. In the previous study, only beef and lamb samples were clearly discriminated from the other groups when representing LV1 vs. LV2, whereas the remaining species showed significant overlap, particularly chicken, turkey, rabbit and quail. In contrast, the current approach using the 7 to 17 minutes segment achieved more distinct discrimination and improved separation for most species, including quail and duck, which appeared better resolved compared to the previous results.

In terms of classification performance, Table 4 compares the cross-validation sensitivity, specificity, and classification errors for each method. Compared to the previous study results, the proposed method achieved higher specificity for all the meat species and reduced classification errors for beef, duck, pork and quail samples. Specifically, the preceding study's approach reported a

classification error of 2% for quail, while the current method reduced this to 0.36%. Similarly, chicken samples in the first study showed a specificity of 98.6%, whereas the proposed method achieved 99.3%. These improvements highlight the potential of focusing on the 7 to 17 minutes segment to enhance classification accuracy while simplifying data analysis.

These results confirm the effectiveness of the proposed HPLC-UV fingerprinting methodology (280 nm, 7 to 17 minutes segment) for classifying meat samples based on their animal species. The fingerprints, obtained from a straightforward water-based extraction, provide reliable chemical descriptors for assessing both classification and authenticity of meat products based on the animal species.

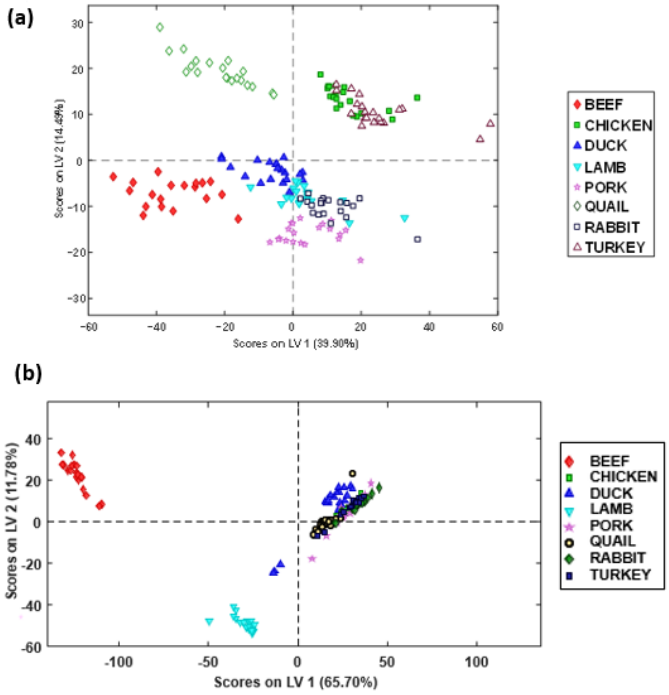

Figure 6. Partial least squares-discriminant analysis (PLS-DA) score plots of (a) LV1 *vs.* LV2 when employing HPLC-UV fingerprints (280 nm, segment from 10 to 17 min) as sample chemical descriptors of the analysed meat samples. The PLS-DA model was built by employing 7 LVs. And (b) LV1 *vs.* LV2 when employing HPLC-UV fingerprints (280 nm, full chromatographic fingerprint) as sample chemical descriptors of the analysed meat samples. The PLS-DA model was built by employing 7 LVs. Data reproduced from ref. [34].

Table 4. PLS-DA cross-validated multiclass predictions for the set of meat samples based on animal species. Comparison between results from the previous study conducted within the research group [34] and the current study.

Sample class variety	Sensitivity (%)		Specificity (%)		Accuracy (classification error, %)	
	Previous Study [34]	Current Study	Previous Study [34]	Current Study	Previous Study [34]	Current Study
Beef	100	100	99.3	100	0.4	0
Chicken	95.0	100	98.6	99.3	0.3	0.36
Duck	100	100	99.3	100	0.4	0
Lamb	100	100	100	100	0	0
Pork	100	100	99.3	100	0.4	0
Quail	100	99.3	95.7	99.3	2	0.36
Rabbit	100	100	100	100	0	0
Turkey	100	100	100	100	0	0

Finally, although the multiclass PLS-DA model showed excellent classification performance for the eight meat species under study, its prediction capability was further evaluated using a hierarchical classification decision tree. This decision tree consisted of consecutive dual PLS-DA models built following the procedure described in section 5.4.1. For this analysis, 30% of the samples were randomly selected from each class (48 samples in total) and used as unknowns for prediction, while the remaining 112 samples were employed to build the paired PLS-DA models at each node of the tree. The tree structure progressed as follows: (1) beef vs. others, (2) duck vs. others (excluding beef), (3) pork vs. others (excluding beef and duck), (4) chicken vs. others (excluding beef, duck, and pork), (5) lamb vs. others (excluding beef, duck, pork, and chicken), (6) quail vs. others (excluding beef, duck, pork, chicken, and lamb), and (7) rabbit vs. turkey (excluding beef, duck, pork, chicken, lamb and quail). Both the classification decision tree diagram and the confusion matrix summarizing the results for prediction and accuracy for each meat species under study are presented in the appendix 1.

The decision tree achieved a 100% accuracy in classifying the 48 unknown samples, significantly enhancing the performance observed with the conventional multiclass PLS-DA model. This additional analysis further validates the robustness and precision of the proposed methodology for meat species classification.

6.2. Classification of Meat Samples based on Different Meat Attributes (Production Systems, Geographical Origin, Halal or Kosher Meats)

6.2.1. HPLC-UV Fingerprints of Meat Samples Based on Different Meat Attributes

The classification and authentication of meat samples according to various attributes such as production systems, geographical origin, and religious practices is essential for ensuring meat authenticity and quality. In contrast to the authentication of the meat species, these cases cannot be authenticated by means of genetics, so untargeted metabolomic fingerprinting strategies as the one presented in this project may be a good option. Thus, in this section, the obtained HPLC-UV fingerprints for the analysis of meat samples with different attributes, including conventional and organic beef, Halal and Kosher beef, conventional and organic chicken, conventional and PGI lamb, and Halal lamb will be presented. The objective of this study is to identify potential chemical differences in the fingerprints that could serve as discriminant descriptors for classification purposes.

The fingerprints were obtained following the standardized protocol described in Section 5.4.1. Samples of each meat type and their respective attribute variations were analysed using HPLC-UV at 280 nm, focusing on the chromatographic segment between 7 and 17 minutes. Again, this segment was selected due to its rich metabolite content, which has been shown to provide key information for sample classification.

Figures A2 to A6, presented in Appendix 2, display the comparative fingerprints for each meat type and its attribute variant. Distinct differences were observed in the peak profiles and intensities among the samples, that will be briefly described above.

Chicken vs. Organic Chicken (Figure A2): The most pronounced differences appeared in the region between 9 and 11 minutes, with organic chicken showing higher peak intensities. Additionally, subtle variations were present between 13 and 15 minutes, suggesting compositional differences due to organic breeding methods.

Lamb vs. PGI Lamb (Figure A3): PGI lamb samples showed distinct differences in the region from 9 to 11 minutes, one peak with slightly higher intensity appeared compared to conventional lamb samples which showed two less intense peaks. These differences may be related to geographical or feeding factors associated with PGI certification.

Lamb vs. Halal Lamb (Figure A4): In the case of Halal lamb, one peak appeared in the region from 9 to 11 minutes and two less intense peaks appeared in the same region in conventional lamb. Additionally, minor variations in peak shapes and intensities were observed between 13 and 15 minutes.

Beef vs. Organic Beef (Figure A5): Differences in the fingerprints were primarily observed in the region from 9 to 11 and 13 to 15 minutes, where both organic beef samples showed distinctions in the peaks. These variations could be attributed to differences in feeding practices or production systems.

Beef vs. Halal and Kosher Beef (Figure A6): The Halal and Kosher beef samples exhibited notable differences in the region between 9 and 11 minutes, where additional peaks in Kosher Beef and less peaks in Halal Beef were observed compared to conventional beef. Furthermore, the peaks between 13 and 15 minutes showed variations, potentially indicating differences in metabolite composition due to the slaughtering process.

These observations highlight the potential of HPLC-UV fingerprinting for distinguishing meat samples based on their attributes. The differences in peak profiles and intensities indicate variations in the metabolite composition, which can serve as valuable descriptors for classification purposes. In the following sections, these fingerprints will be used as input data for chemometric analyses to assess their classification potential.

6.2.2. Meat Classification based on Different Meat Attributes (Production Systems, Geographical Origin, Halal or Kosher Meats) by Partial Least Squares-Discriminant Analysis

As demonstrated in previous analyses, the HPLC-UV fingerprints obtained within the chromatographic segment from 7 to 17 minutes provide excellent chemical descriptors for classifying meat samples based on animal species. However, this authentication challenge can easily be addressed using genetic-based methods, while other meat authenticity concerns, particularly those related to attributes highly valued by society today, such as production systems, geographical origin, and religious practices, cannot be easily identified through genetic analysis, as commented in the introduction. The potential of the obtained HPLC-UV fingerprints to address these authenticity issues was evaluated by employing chicken, lamb, and beef meats, based on sample availability. Before performing the PLS-DA analysis, the obtained HPLC-UV fingerprints (7 to 17-minute segment) were initially subjected to PCA to evaluate the behaviour of the QC samples and confirm the robustness and reproducibility of the methodology. In all cases, the QCs appeared tightly clustered, validating the reliability of the proposed approach. Once the robustness was confirmed, the PLS-DA models were developed.

Chicken samples were analysed to evaluate authenticity related to organic production. For this purpose, 20 conventional and 20 organic chicken samples (all from Catalonia, Spain) were analysed using the proposed methodology, and the obtained HPLC-UV fingerprints (segment from 7 to 17 minutes) were subjected to PLS-DA. Figure 7a shows the obtained PLS-DA score plot of LV1 vs. LV2. This model was built using 2 latent variables (LVs) and it shows perfect discrimination between the two groups based on LV1, with sensitivity, specificity, and accuracy values of 100%. These results highlight the ability of the proposed methodology to discriminate between chicken samples based on their production system, likely due to differences in feeding practices and rearing environments.

For lamb samples, three classes were analysed: lamb meat produced in Catalonia (Spain), PGI lamb meat produced in Aragon (Spain), and Halal lamb meat produced in Catalonia (Spain). A total of 20 samples per class were subjected to the proposed methodology, and the resulting PLS-DA score plot of LV1 vs. LV2 (built using 4 LVs) depicted in Figure 7b demonstrated excellent discrimination among the three sample groups. LV1 allowed the separation of conventional lamb (negative LV1 values) from the other two groups, while LV2 differentiated Halal lamb samples (negative LV2 values) against the other two groups. Sensitivity, specificity, and accuracy values of 100% were achieved for all three classes, demonstrating the capability of the proposed methodology to address both geographical origin and religious production system issues in lamb samples.

In the case of beef, five sample classes were analysed (all of them produced in Catalonia (Spain)): conventional beef, two organic beef types (labelled as Organic Beef 1 and Organic Beef 2), Halal beef, and Kosher beef. The obtained HPLC-UV fingerprints (segment from 7 to 17 minutes) were subjected to PLS-DA analysis, and the resulting score plot of LV1 vs. LV2 vs. LV3 (built with 3 LVs) represented in Figure 7c, showed a very acceptable sample discrimination among the five beef sample groups under study.

PLS-DA multiclass prediction values by cross-validation for the set of beef meat samples are summarized in Table 5. Calibration and cross-validation sensitivity values were higher than 95%, while specificity values also exceeded 95% for calibration and were higher than 91.2% for cross-validation. Although accuracy was slightly lower compared to the previously discussed examples, classification errors remained below 5% for calibration and 6.9% for cross-validation, which are still acceptable given the types of samples analysed.

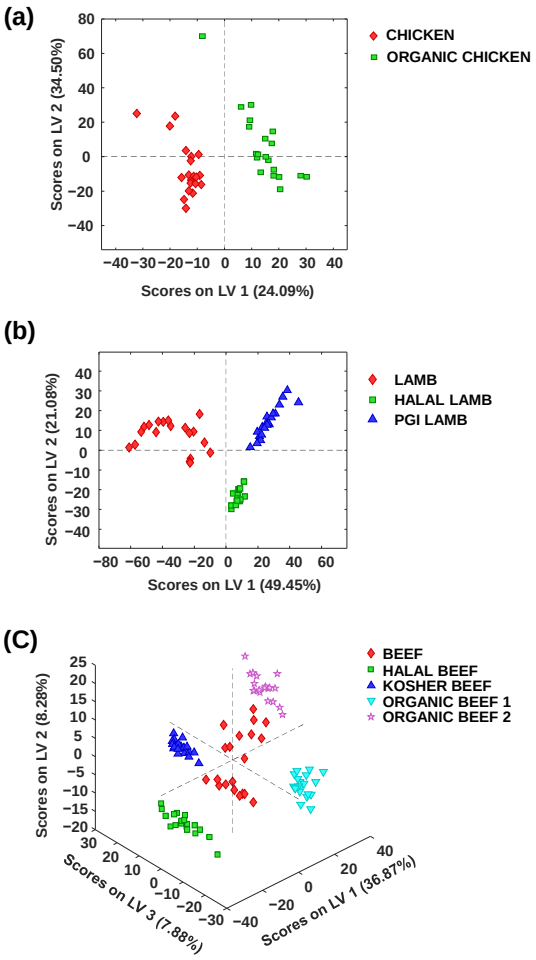

Figure 7. Partial least squares-discriminant analysis (PLS-DA) score plots when employing HPLC-UV fingerprints to address classification of meat samples based on different meat production attributes. (a) chicken samples, (b) lamb samples, and (c) beef samples.

Table 5. PLS-DA multiclass predictions by cross-validation for the set of beef meat samples.

Sample class variety	Sensitivity (%)		Specificity (%)		Accuracy (classification error, %)	
	Calibration	Cross-validation	Calibration	Cross-validation	Calibration	Cross-validation
Beef	95.0	95.0	95.0	91.2	5	6.9
Halal Beef	100	100	100	100	0	0.36
Kosher Beef	100	100	100	100	0	0
Organic Beef 1	100	100	98.8	97.5	0.6	1.3
Organic Beef 2	100	95.0	100	100	0	2.5

In any case, when the specific attributes under study, such as organic vs. conventional beef or Halal/Kosher vs. conventional beef, were analysed separately, the classification performance improved significantly, as illustrated in Figure 8. This figure shows the PLS-DA plots comparing conventional beef with organic beef (Figure 8a) and with Halal and Kosher beef (Figure 8b). In addition, as seen in Table 6, for organic beef production, sensitivity, specificity, and accuracy values reached 100%, except for cross-validation, where specificity exceeded 95.0% and accuracy was above 97.5%. In contrast, Table 7 shows that Halal and Kosher beef production achieved perfect classification, with sensitivity, specificity, and accuracy values of 100% across all sample groups.

Overall, the results demonstrate that the proposed HPLC-UV fingerprints at 280 nm (7 to 17 minutes segment), obtained through a simple meat water sonication extraction, serve as excellent chemical descriptors for addressing meat authenticity issues. Combined with PLS-DA, this methodology proves highly effective for classifying meat samples based on attributes such as production systems, geographical origin, and religious practices, establishing itself as a robust method for ensuring meat authenticity and quality assurance.

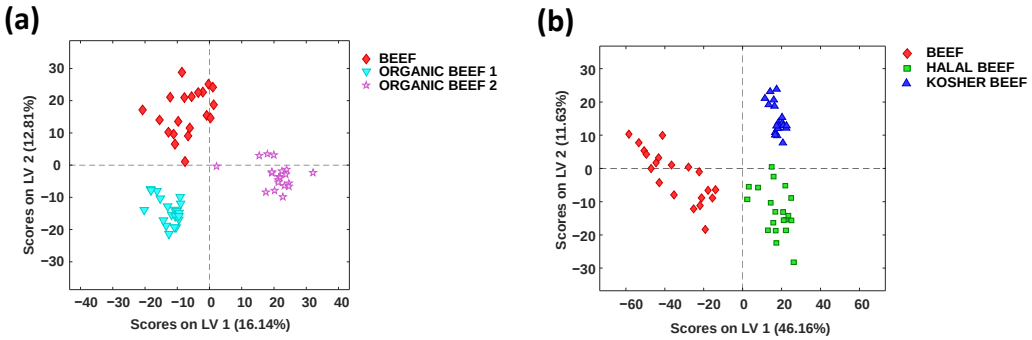

Figure 8. PLS-DA score-plots of LV1 *vs.* LV2 when employing HPLC-UV fingerprints (280 nm, segment from 7 to 17 min) as sample chemical descriptors for the classification of meat samples of (a) beef vs. organic beefs (model built employing 3 LVs) and (b) beef vs. Halal and Kosher beefs (model built employing 5 LVs).

Table 6. PLS-DA multiclass predictions by cross-validation for the set of organic beef meat samples.

Sample class variety	Sensitivity (%)		Specificity (%)		Accuracy (classification error, %)	
	Calibration	Cross-validation	Calibration	Cross-validation	Calibration	Cross-validation
Beef	100	100	100	100	0	0
Organic Beef 1	100	100	100	100	0	0
Organic Beef 2	100	95.0	100	100	0	2.5

Table 7. PLS-DA multiclass predictions by cross-validation for the set of religious beef meat samples.

Sample class variety	Sensitivity (%)		Specificity (%)		Accuracy (classification error, %)	
	Calibration	Cross-validation	Calibration	Cross-validation	Calibration	Cross-validation
Beef	100	100	100	100	0	0
Halal Beef	100	100	100	100	0	0
Kosher Beef	100	100	100	100	0	0

6.2.3. Detection and Quantitation of Meat Adulteration Frauds by Partial Least Squares Regression

The potential of the proposed HPLC-UV fingerprints to detect and quantify meat adulterations based on specific meat attributes was evaluated using PLS regression. Six adulteration cases were studied: (i) PGI lamb (produced in Aragon, Spain) adulterated with lamb (produced in Catalonia, Spain), (ii) organic chicken adulterated with non-organic chicken, (iii) organic beef adulterated with non-organic beef, (iv) Halal lamb adulterated with non-Halal lamb, (v) Halal beef adulterated with non-Halal beef, and (vi) Kosher beef adulterated with non-Kosher beef.

For PLS regression, two sets of adulterated blended meat samples were prepared: one for training and another for validation/prediction. The training set included adulteration levels of 0% (pure studied meat), 20%, 40%, 60%, 80%, and 100% (pure adulterant meat). The validation/prediction set consisted of samples with adulteration levels of 15%, 25%, 50%, 75%, and 85%. Each

adulteration level was prepared in quintuplicate. Additionally, a 50% adulteration level was included in each case as a quality control (QC) to ensure robustness and reproducibility of the PLS predictions. All adulterated samples were analysed in a random order, with QC samples injected every 10 sample injections.

Initially, the obtained HPLC-UV fingerprints (7 to 17 minutes segment) were subjected to PCA to assess the behaviour of the QCs and visualize the distribution of adulteration levels in the PC1 vs. PC2 plot. In all cases, the QCs appeared clustered together, confirming the robustness and reproducibility of the methodology. Furthermore, the samples were distributed along PC1 based on their adulteration content, with pure samples positioned at opposite ends of PC1.

PLS regression was then performed for each adulteration case, and the resulting Y predicted vs. Y measured plots are shown in Figure 9. The performance metrics for the six meat adulteration cases are summarized in Table 8. Overall, the results demonstrated very acceptable performance for the detection and quantitation of meat adulteration frauds involving different production systems. Good calibration linearity was observed in all cases, with R^2 values ranging from 0.928 to 0.998 for the calibration scatter plots of predicted vs. measured adulteration levels. Calibration errors below 9% and 10.6% for calibration and cross-validation, respectively, were obtained. Besides, prediction calibration errors between 2.4 and 6.6% were achieved, demonstrating the feasibility of the proposed methodology to detect and quantify adulterations in meat products adulterated with other meat matrices, even in cases based on meat quality attributes. Moreover, the simplicity of the methodology, based on water sonication and HPLC-UV analysis, offers an economical and accessible approach for food quality laboratories, eliminating the need for expensive instrumentation like LC-MS or LC-HRMS or the targeting of specific metabolites. This fingerprinting approach provides an efficient and cost-effective solution for addressing meat authenticity concerns.

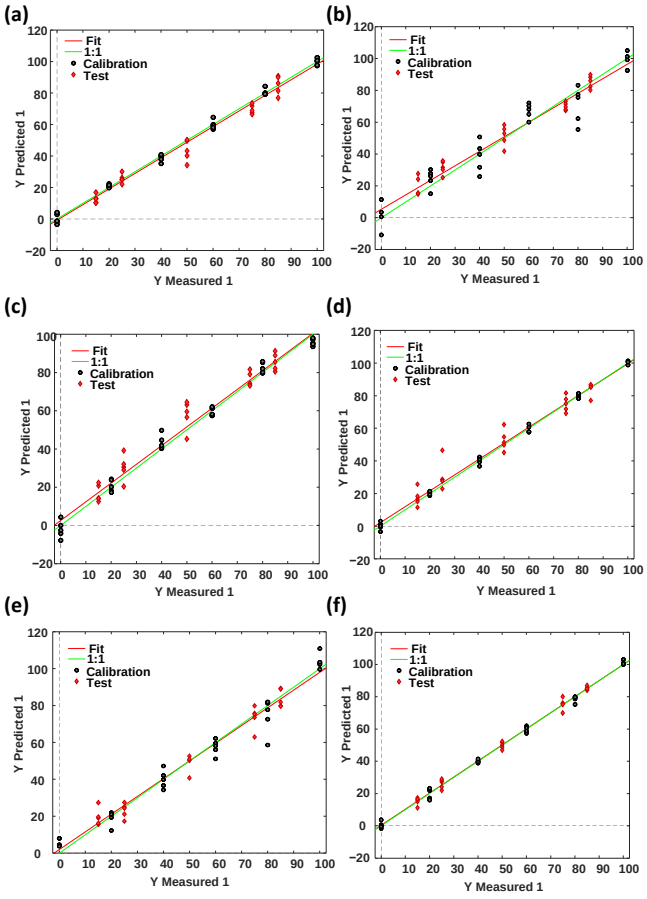

Figure 9. Partial least squares (PLS) regression results depicting the scatter plots of predicted vs. measured percentages when evaluating different meat adulteration cases. (a) PGI lamb adulterated with conventional lamb, (b) organic chicken adulterated with non-organic chicken, (c) organic beef adulterated with non-organic beef, (d) Halal lamb adulterated with non-Halal lamb, (e) Halal beef adulterated with non-Halal beef, and (f) Kosher beef adulterated with non-Kosher beef.

Table 8. Evaluation of meat adulterations by PLS using HPLC-UV fingerprints as chemical descriptors.

Adulteration case*	LVs	Linearity (R^2)	Calibration error (%)	Cross-validation error (%)	Prediction error (%)
PGI lamb	4	0.996	2.2	4.1	5.7
Organic chicken	2	0.928	9.0	10.0	6.1
Organic beef	3	0.987	3.9	10.6	6.6
Halal lamb	6	0.998	1.7	5.8	6.4
Halal beef	2	0.966	6.2	7.9	5.2
Kosher beef	5	0.997	2.0	3.9	2.4

*conventional chicken, lamb or beef were employed as adulterant, depending on the adulteration case.

7. CONCLUSIONS

The results obtained in this study demonstrate that a relatively simple technique, widely available in most laboratories, such as liquid chromatography with ultraviolet detection, can be effectively used for non-targeted metabolomic analysis to generate HPLC-UV fingerprints. These fingerprints have proven to be excellent chemical descriptors for meat authentication and the detection of meat fraud. The study focused on analysing the chromatographic segment between 7 and 17 minutes, which contained the majority of key metabolites responsible for differentiating the analysed samples. By analysing the differences in metabolite profiles and relative signal abundances, it was possible to successfully classify meat samples according to their species, geographical origin, and production system, including organic, Halal, and Kosher products. These findings highlight the robustness and practicality of HPLC-UV fingerprinting as a tool for addressing authenticity issues in the meat industry.

In all the studies performed, the analysis of QCs through PCA demonstrated the reproducibility and robustness of the obtained data and the proposed methodology.

The PLS-DA analysis confirmed the high discriminatory power of the proposed method, achieving sensitivity and specificity values of 100% and higher than 99.3% for calibration and cross-validation, respectively. The classification performance was further validated with unknown samples by means of a classification decision tree using HCA, achieving an impeccable 100% prediction accuracy when 48 unknown samples were considered. These results emphasize the reliability of this methodology in addressing complex authentication challenges beyond the capabilities of traditional genetic methods.

An additional strength of this approach is its ability to discriminate not only between different species but also within the same species based on specific attributes, such as geographical origin or production system. For example, the method achieved perfect separation between organic and non-organic chicken, as well as among lamb, Halal lamb, and PGI lamb. For beef samples, which included conventional, organic, Halal, and Kosher groups, classification errors were kept below 6.9%, demonstrating the robustness of the methodology.

Moreover, the proposed HPLC-UV fingerprints proved to be a valuable tool for detecting and quantifying meat adulteration levels using PLS regression. Six adulteration cases involving PGI, organic, Halal, and Kosher meats were evaluated, yielding calibration, cross-validation, and prediction errors below 9%, 10%, and 6.6%, respectively. These results further validate the suitability of this method for addressing authenticity issues in the meat industry.

In conclusion, HPLC-UV fingerprinting offers a straightforward, cost-effective, and efficient solution for meat authentication, addressing key industry challenges such as species fraud, geographical origin, and production systems. This methodology not only provides reliable results but also contributes to improving quality control and preventing fraudulent practices in the food industry.

8. FUTURE STUDIES

One of the key advantages of the proposed methodology is its ability to authenticate meat samples without requiring the identification of specific discriminant metabolites, as it relies solely on a fingerprinting strategy. This simplicity enhances the efficiency and practicality of the method, making it applicable in a wide range of scenarios. However, exploring the chemical composition of the most discriminant metabolites could provide additional insights and improve the method's robustness.

Future research will prioritize analysing the loadings plots derived from PLS-DA models to identify the metabolites that contribute most significantly to the differentiation of meat samples. These key metabolites will then be characterized and identified using advanced mass spectrometry techniques, including both low- and high-resolution mass spectrometry.

The identification of discriminant metabolites aims to establish them as potential biomarkers for meat authenticity. This would not only validate the current fingerprinting approach but also serve as a basis for designing more targeted and rapid authentication methods. Such advancements would strengthen quality control processes and enhance the ability to detect fraudulent practices in the meat industry.

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12. ACRONYMS

CV: Cross-Validation

DNA: Deoxyribonucleic Acid

EU: European Union

FN: False Negative

FP: False Positive

HCA: Hierarchical Cluster Analysis

HMB: Hierarchical Model Builder

HPLC: High-Performance Liquid Chromatography

LC-HRMS: Liquid Chromatography-High-Resolution Mass Spectrometry

LC-MS: Liquid Chromatography Mass Spectrometry

LV: Latent Variable

MLR: Multiple Linear Regression

NMR: Nuclear Magnetic Resonance

ODS: Objetivos de Desarrollo Sostenible (Sustainable Development Goals)

OFPA: Organic Foods Production Act

PC: Principal Component

PCA: Principal Component Analysis

PCR: Polymerase Chain Reaction

PDO: Protected Designation of Origin

PGI: Protected Geographical Indication

PLS: Partial Least Squares

PLS-DA: Partial Least Squares-Discriminant Analysis

PTFE: Polytetrafluoroethylene

QC: Quality Control

SDG: Sustainable Development Goals

TN: True Negative

TP: True Positive

TS: Total Samples

TSG: Traditional Specialities Guaranteed

UAE: Ultrasound-Assisted Extraction

UHPLC: Ultra-High-Performance Liquid Chromatography

USA: United States of America

UV: Ultraviolet

WPALS: Well Plate Autosampler

APPENDICES

APPENDIX 1: HIERARCHICAL CLASSIFICATION DECISION TREE AND CONFUSION MATRIX FOR MEAT SPECIES PREDICTION

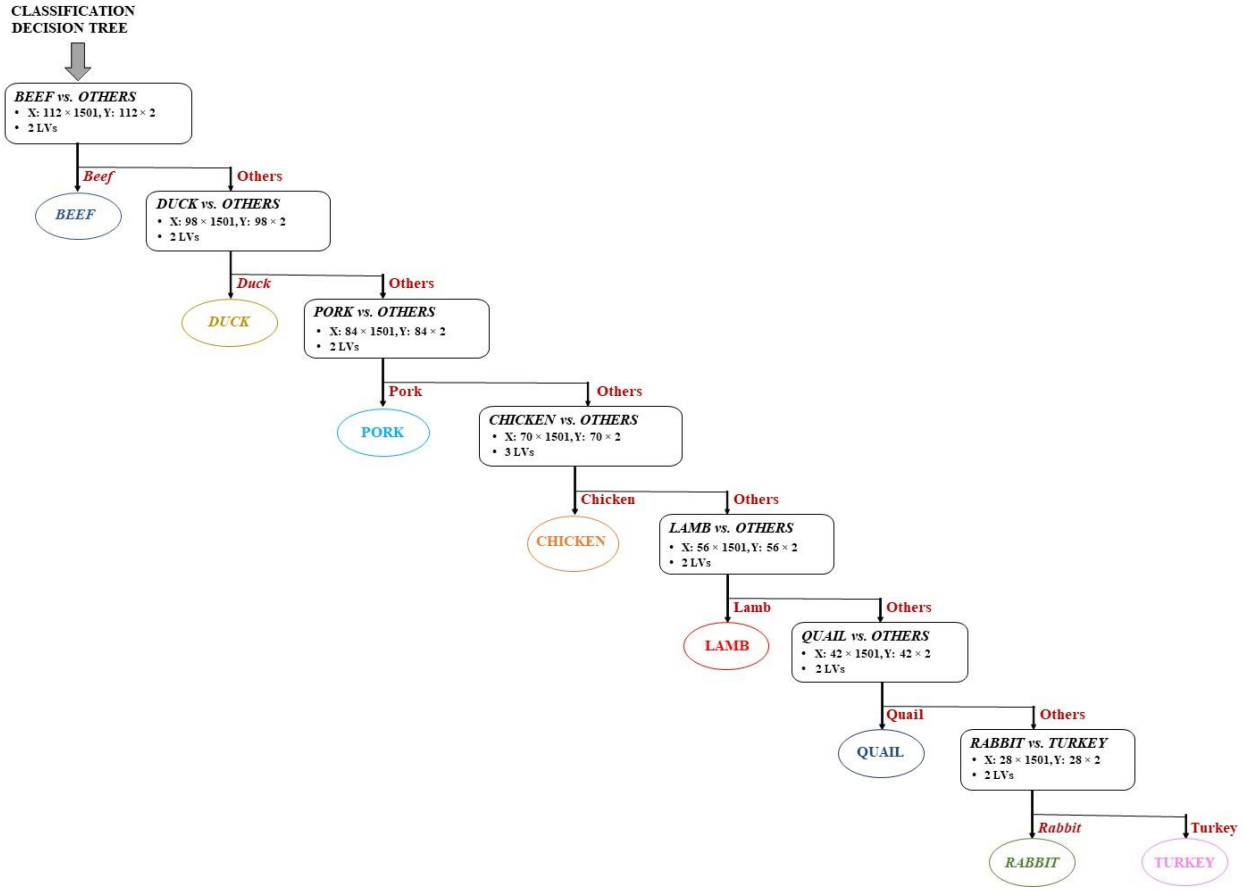

Figure A1. Flowchart of the classification decision tree constructed using PLS-DA models as the rule nodes. The dimensions of the data matrices and LVs employed to build the paired PLS-DA calibration models are indicated.

Table A1. Prediction confusion matrix for animal meat species when using a classification decision tree based on dual PLS-DA models.

	Beef	Chicken	Duck	Lamb	Pork	Quail	Rabbit	Turkey	Accuracy (%)
Beef	6	0	0	0	0	0	0	0	100
Chicken	0	6	0	0	0	0	0	0	100
Duck	0	0	6	0	0	0	0	0	100
Lamb	0	0	0	6	0	0	0	0	100
Pork	0	0	0	0	6	0	0	0	100
Quail	0	0	0	0	0	6	0	0	100
Rabbit	0	0	0	0	0	0	6	0	100
Turkey	0	0	0	0	0	0	0	6	100

APPENDIX 2: HPLC-UV FINGERPRINTS OF MEAT SAMPLES BASED ON DIFFERENT ATTRIBUTES

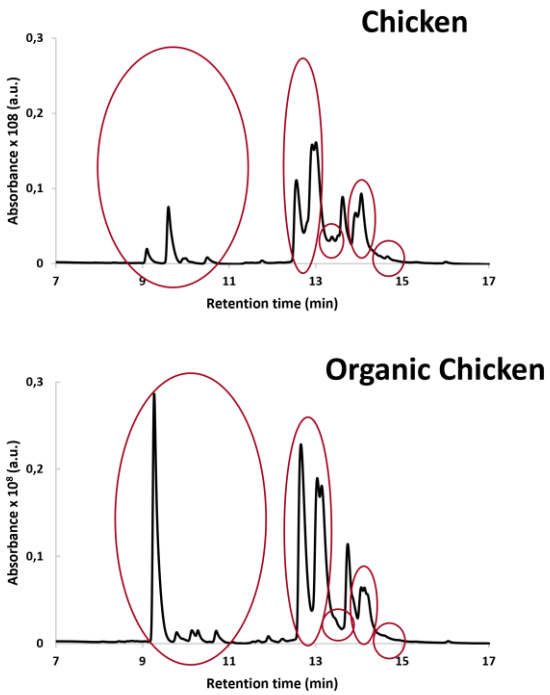

Figure A2. HPLC-UV fingerprints (at 280 nm, segment from 7 to 17 min) for one selected sample of conventional and organic chicken meat and its comparison.

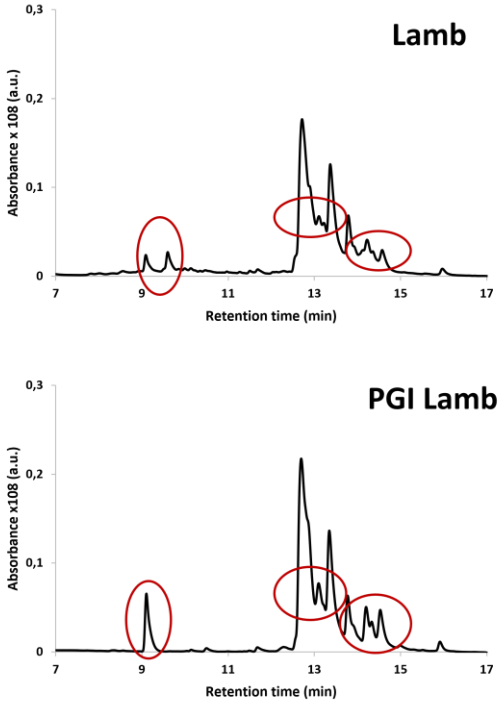

Figure A3. HPLC-UV fingerprints (at 280 nm, segment from 7 to 17 min) for one selected sample of conventional and PGI lamb meat and its comparison.

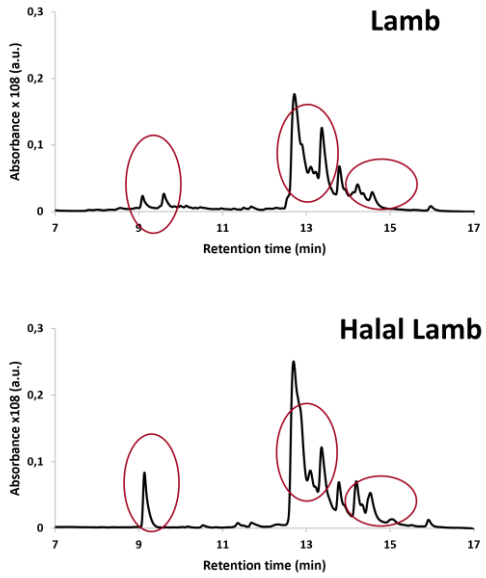

Figure A4. HPLC-UV fingerprints (at 280 nm, segment from 7 to 17 min) for one selected sample of conventional and Halal lamb meat and its comparison.

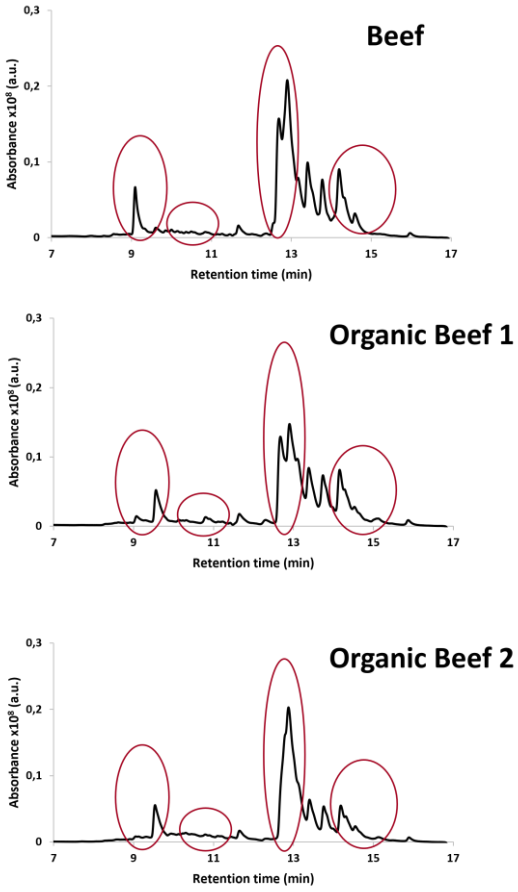

Figure A5. HPLC-UV fingerprints (at 280 nm, segment from 7 to 17 min) for one selected sample of conventional and two different organic beef meat and its comparison.

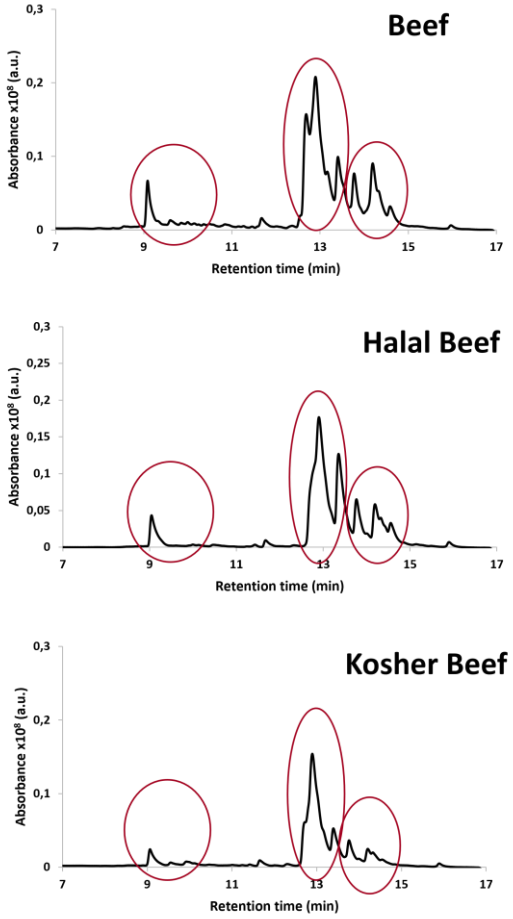

Figure A6. HPLC-UV fingerprints (at 280 nm, segment from 7 to 17 min) for one selected sample of conventional, Halal and Kosher beef meat and its comparison.