INNOVATIVE TOOLS FOR ENHANCING AUTHENTICITY AND TRACEABILITY IN THE FOOD SUPPLY CHAIN

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Doctoral Dissertation
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INOVATIVNA ORODJA ZA IZBOLJŠANJE PRISTNOSTI IN SLEDLJIVOSTI V VERIGI PRESKRBE S HRANO

Doktorska disertacija

Supervisor: Prof. Dr. Nives Ogrinc

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"The struggle itself toward the heights is enough to fill a man's heart. One must imagine Sisyphus happy."
— Albert Camus, The Myth of Sisyphus (1942)

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Abstract

Food fraud has been a persistent issue for decades, transforming from basic adulteration into a sophisticated, multibillion-dollar problem driven by significant economic incentives. With the global economic impact on the food industry estimated at USD 30–65 billion annually and over 10% of the world's food supply affected each year, the consequences are far-reaching. Beyond financial losses, food fraud undermines consumer trust, damages the reputation of legitimate producers, and imposes heavy regulatory burdens, positioning fraud prevention as one of the most urgent and complex challenges confronting modern global food systems.

This doctoral dissertation addresses the critical need for strengthening food authenticity and traceability by introducing a novel, integrated framework comprising three complementary systems: a Physical Traceability System that relies on advanced analytical techniques and database-driven authentication; a Rapid Traceability System, enabling fast and reliable spectroscopic screening for fraud detection; and a Digital Traceability System, which employs cutting-edge Internet of Things (IoT) technologies to provide real-time, continuous monitoring across the entire supply chain.

The framework adopts a multi-disciplinary approach comprising (1) The application of stable isotope ratios and multi-elemental analyses to distinguish the geographical origin and production methods (conventional vs organic) of spices, (2) the use of FTIR spectroscopy to support the detection of adulteration and authenticity, (3) expanding the IsoFoodTrack authentication database by incorporating new isotopic, elemental, and metadata entries, (4) employing advanced chemometric techniques such as OPLS-DA and DD-SIMCA and AI models to interpret complex datasets, and (5) implementing IoT-enabled digital traceability for seafood supply chains through a pilot project in Montenegro. This approach is applied to four high-value commodities: saffron, paprika, cinnamon, and seafood, which were selected due to their high susceptibility to fraud.

The work yielded several key findings. The IsoFoodTrack database demonstrated a scalable and innovative framework for integrating isotopic, elemental, and related metadata to verify authenticity and ensure traceability. This architecture enabled the continuous inclusion of new analytical metrics and food categories, which strengthened its effectiveness in fraud detection over time. Authentic saffron samples achieved 94% classification accuracy for geographical-origin authentication using isotopic and elemental analyses, with DD-SIMCA identifying potential mislabeling in up to 15% of commercial samples, particularly those labelled as Iranian. For paprika and cinnamon, accuracies of 90% and 89%, respectively, were achieved. Differentiation by agricultural production methods was achieved with ~95% accuracy for cinnamon, while for paprika, characteristic isotopic (δ^{15}N, δ^{34}S) and elemental (Zn) markers enabled reliable separation of production types. FTIR spectroscopy further distinguished Ceylon from cassia cinnamon and highlighted possible paprika adulteration. Applying artificial intelligence modeling, specifically the

Light Gradient Boosting Machine (LightGBM), to 115 saffron samples achieved an overall classification accuracy of 93% using combined isotopic and elemental data. SHapley Additive exPlanations (SHAP) analysis identified the most influential elemental discriminators Ni, Co, K, Mn, Sr, Rb, Ga, Al, Cd, and Pb, enabling a targeted analytical approach that could reduce analysis costs by approximately 79% while maintaining classification accuracies above 90%. The IoT-enabled system was designed to facilitate real-time monitoring of seafood supply chains by integrating environmental sensors (water temperature, dissolved oxygen, pH, and salinity) with GPS and onboard sensors for transport conditions, all managed via Cloud-based data. QR codes will enable interactions with users and stakeholders outside the fish farm, providing product/package information and collecting their feedback.

The integrated traceability framework presented in this thesis provides a highly effective approach for authenticating and tracing high-value commodities such as saffron, paprika, cinnamon, and seafood. Through its advanced capabilities in detecting geographical origins, production methods, and adulteration, it proves a powerful tool in combating food fraud. Future work should broaden geographical and commodity scope via international collaborations and open-data platforms, incorporate explainable AI (XAI) for transparent decisions, and combine compound-specific isotope analysis (CSIA) with metabolomics to further improve analytical precision. Policymakers should promote AI, machine learning, and blockchain integration into existing traceability systems to boost transparency and fraud prevention.

Povzetek

Prevare s hrano že desetletja predstavljajo trdovraten in vse bolj zapleten problem, ki se je iz preprostih oblik ponarejanja razvil v sofisticirano, večmilijardno globalno dejavnost, podprto z močnimi ekonomskimi interesi. Letna gospodarska škoda v prehranski industriji je ocenjena na 30 do 65 milijard ameriških dolarjev, pri čemer je vsako leto prizadetih več kot 10 % svetovne oskrbe s hrano; posledice pa segajo daleč onkraj neposrednih finančnih izgub, saj lahko tovrstne prevare resno ogrozijo tudi zdravje potrošnikov ter povečajo tveganje za izpostavljenost neustreznim, nekakovostnim ali celo nevarnim živilom.

Predlagana doktorska disertacija uvaja nov, integriran sistem za spremljanje pristnosti in sledljivosti živil in obsega: fizično sledljivost, ki temelji na naprednih analitskih tehnikah in preverjanju na osnovi podatkovne zbirke pristnih vzorcev; hitre sisteme, ki omogočajo hitro in zanesljivo odkrivanje prevar na osnovi spektroskopskih metod; ter digitalni sistem, ki uporablja najnaprednejše tehnologije interneta stvari (IoT) za zagotavljanje neprekinjenega spremljanja parametrov v realnem času po celotni dobavni verigi.

Doktorska disertacija zajema večdisciplinarni pristop, in sicer (1) uporabo razmerij stabilnih izotopov lahkih elementov in multielementno analizo za razlikovanje geografskega porekla in načina pridelave (konvencionalno proti organskemu) začimb; (2) uporabo FTIR spektroskopije za podporo pri odkrivanju in preverjanju pristnosti začimb; (3) razširitev podatkovne zbirke IsoFoodTrack z novimi izotopskimi, elementnimi in metapodatki; (4) uporabo naprednih kemometrijskih tehnik, kot sta OPLS-DA in DD-SIMCA, ter modelov umetne inteligence za interpretacijo kompleksnih podatkovnih naborov in (5) implementacijo IoT-podprte digitalne sledljivosti za dobavne verige z morsko hrano (ribe) prek pilotnega projekta v Črni gori. Ta pristop smo preizkusili na izbranih vrstah živil, ki so najbolj izpostavljena potvorbam, in sicer na začimbah (žafran, paprika in cimet) ter ribah.

Delo je pomembno prispevalo k oblikovanju uporabnih in praktično relevantnih zaključkov. Podatkovna zbirka IsoFoodTrack predstavlja edinstveno in trenutno najsodobnejšo zbirko, ki temelji na skalabilni in inovativni arhitekturi za integracijo izotopskih, elementnih ter sorodnih metapodatkov, namenjenih preverjanju pristnosti in zagotavljanju sledljivosti živil. Takšna arhitektura omogoča vključevanje novih analitskih podatkov in kategorij živil, s čimer se njena učinkovitost pri odkrivanju potvorb živil še dodatno krepi. Nadalje smo z uporabo izotopskih parametrov in elementne sestave preverili geografsko poreklo žafrana s 94 % natančnostjo klasifikacije. Z uporabo modela DD-SIMCA smo pri 15 % komercialnih vzorcev zaznali morebitno napačno označevanje, pri čemer so bile nepravilnosti najpogosteje ugotovljene pri vzorcih iz Irana. Za papriko in cimet smo dosegli 90 % oziroma 89 % natačnost pri preverjanju geografskega porekla, medtem ko smo za razlikovanje načina pridelave dosegli 95 % natančnost za cimet. Nadalje

smo ugotovili, da so najbolj značilni parametri za razlikovanje med konvencionalno in organsko pridelano paprikoizotopski parametri ($\delta^{15}N$, $\delta^{34}S$) in element Zn. S pomočjo FTIR spektroskopije smo uspešno razlikovali cejlonski cimet od kasije ter identificirali morebitne potvorbe paprike. Obdelavo podatkov žafrana smo nadalje nadgradili z uporabo umetne inteligence, natančneje Light Gradient Boosting Machine (LightGBM). Na naboru 115 vzorcev žafrana smo z uporabo kombiniranih izotopskih in elementnih podatkov dosegli visoko natančnost klasifikacije glede na geografsko poreklo, in sicer 93 %. Analiza SHapley Additive exPlanations (SHAP) je razkrila najvplivnejše diskriminatorne parametre (Ni, Co, K, Mn, Sr, Rb, Ga, Al, Cd in Pb), kar omogoča razvoj ciljno usmerjenega analitskega pristopa in analizo izbranih elementov. Tak pristop lahko zmanjša stroške analiz za približno 79 %, hkrati pa ohrani natančnost klasifikacije nad 90 %. IoT-podprti sistem je bil zasnovan za spremljanja dobavnih verig rib v realnem času z integracijo okoljskih senzorjev (temperature vode, koncentracije raztopljenega kisika, pH in slanosti) ter z GPS in temperaturnimi senzorji za spremljanje pogojev transporta. QR-kode bodo omogočale interakcijo z uporabniki in drugimi deležniki zunaj ribogojnice, saj bodo zagotavljale informacije o izdelkih oziroma pakiranjih ter hkrati omogočale zbiranje njihovih povratnih informacij.

Integrirani sistem sledljivosti, predstavljen v tej disertaciji, predstavlja možnost učinkovitega pristopa za preverjanje pristnosti in sledenje visoko kakovostnim izbranim vrstam živil, ki so izpostavljena potvorbam, kot so žafran, paprika, cimet in ribe. Sistem, razvit in predstavljen na izbranih vrstah živil v tej disertaciji, je zasnovan modularno in prilagodljivo, kar omogoča njegov učinkovit prenos ter uporabo tudi pri drugih živilih in prehranskih izdelkih.

V prihodnje je smiselno vzpostaviti in nadalje nadgrajevati odprto platformo ter podatkovno zbirko za različne vrste živil, vključiti razložljivo umetno inteligenco (XAI) za bolj pregledne in zaupanja vredne odločitvene procese ter povezati analizo stabilnih izotopov na molekularni ravni z metabolomiko za nadaljnje izboljšanje analitične natančnosti. Na ravni oblikovanja politik pa je priporočljivo spodbujati integracijo umetne inteligence, strojnega učenja ter digitalnih pristopov, kot so internet stvari (IoT) in tehnologija veriženja blokov, v obstoječe sisteme sledljivosti hrane z namenom povečanja preglednosti, zanesljivosti in učinkovitosti odkrivanja prehranskih prevar.

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Abbreviations and Symbols

1H NMR	. . . Proton Nuclear Magnetic Resonance
2H-SNIF NMR	. . . Site-specific natural isotopic fractionation as measured by nuclear magnetic resonance
AA	. . . Antioxidant Activity
C3	. . . C3 plants, Calvin cycle
C4	. . . C4 plants, Hatch-Slack cycle
CAGR	. . . Compound Annual Growth Rate
CRMs	. . . Certified Reference Materials
CEN	. . . European Committee for Standardization
DA	. . . Discriminant Analysis
DART-MS	. . . Direct Analysis in Real Time Mass Spectrometry
DD-SIMCA	. . . Data-Driven Soft Independent Modelling of Class Analogy
DO	. . . Dissolved Oxygen
DPPH	. . . 2,2-Diphenyl-1-picrylhydrazyl
EA-IRMS	. . . Elemental Analyser - Isotope Ratio Mass Spectrometer
EC	. . . Electrical Conductivity
ED-XRF	. . . Energy-Dispersive X-ray Fluorescence
EFSA	. . . European Food Safety Authority
EU	. . . European Union
FAIR	. . . Findable, Accessible, Interoperable and Reusable
FTIR	. . . Fourier-Transform Infrared Spectroscopy
GC	. . . Gas Chromatography
GC-C-IRMS	. . . Gas Chromatography-Combustion Isotope Ratio Mass Spectrometry
GC-IRMS	. . . Gas Chromatography-Isotope Ratio Mass Spectrometry
GC-MS	. . . Gas Chromatography-Mass Spectrometry
GC-P-IRMS	. . . Gas Chromatography-Pyrolysis Isotope Ratio Mass Spectrometry
GMOs	. . . Genetically Modified Organisms
HS-SPME	. . . Headspace-Solid Phase Microextraction
ICP-MS	. . . Inductively Coupled Plasma Mass Spectrometry
IoT	. . . Internet of Things
IRMM	. . . Institute for Reference Materials and Measurements
IRMS	. . . Isotope Ratio Mass Spectrometry
ISO	. . . International Organization for Standardization
IsoVoc	. . . Isotope Volatile Organic Compounds

JSI	. . . Jožef Stefan Institute
LDA	. . . Linear Discriminant Analysis
LightGBM	. . . Light Gradient Boosting Machine
LOD	. . . Limit of Detection
MALDI-TOF-MS	. . . Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry
OCC	. . . One-Class Classification
OPLS-DA	. . . Orthogonal Partial Least Squares Discriminant Analysis
ORP	. . . Oxidation-Reduction Potential
PCA	. . . Principal Component Analysis
PCs	. . . Principal Components
PDO	. . . Protected Designation of Origin
PCR	. . . Polymerase Chain Reaction
PCR-SSCP	. . . Polymerase Chain Reaction–Single Strand Conformation Polymorphism
PNA	. . . Peptide Nucleic Acid
PLS-DA	. . . Partial Least Squares Discriminant Analysis
ppb	. . . Parts per billion
ppm	. . . Parts per million
PTR-MS	. . . Proton Transfer Reaction Mass Spectrometry
SHAP	. . . SHapley Additive exPlanations
SIMCA	. . . Soft Independent Modelling of Class Analogy
SMOTE	. . . Synthetic Minority Over-sampling Technique
RAPD	. . . Random Amplified Polymorphic DNA
SPE	. . . Solid-Phase Extraction
SPME	. . . Solid Phase Microextraction
TPC	. . . Total Phenolic Content
US FDA	. . . United States Food and Drug Administration
VIP	. . . Variable Importance in Projection
VOC	. . . Volatile Organic Compound
VPDB	. . . Vienna Pee Dee Belemnite
VSMOW	. . . Vienna Standard Mean Ocean Water
w/w	. . . Weight Per Weight
wt./vol.	. . . Weight/Volume percentage concentration
δ	. . . Delta, relative isotopic composition, value given in parts per thousand or "permille"
δ^{13}C	. . . Isotopic composition of carbon
δ^{15}N	. . . Isotopic composition of nitrogen
δ^{18}O	. . . Isotopic composition of oxygen
δ^{2}H	. . . Isotopic composition of hydrogen
δ^{34}S	. . . Isotopic composition of sulphur

Chapter 1

Introduction

Imagine walking into a bustling spice market. A small jar of saffron claims to come from Iran, a paprika tin boasts Hungarian origins, and a packet of cinnamon advertises "100% organic." Each product carries a story of geography, tradition, and value—a promise embedded in its label. Yet behind these promises lies an essential question: how can we be sure these stories are true? Today, such markets are no longer confined to local bazaars. They extend into a vast, global network of supply chains that connect farmers, distributors, processors, retailers, and consumers across continents. A single meal might combine ingredients sourced from five—or even ten—different countries: the tomatoes in a salad grown in Spain, the olive oil pressed in Italy, the cheese produced in France, the spices imported from India, and the coffee harvested in Colombia.

This global journey reveals the interconnected nature of modern food systems, which operate across different regions and regulations (Trienkens, 2012). Globalization has shaped these systems into widespread and dependent networks, linking economies and cultures through the movement of goods, people, and information (Ritzer & Dean, 2015; Silvestrini et al., 2023). Population growth and rapid urbanization further drive this transformation. As cities expand, fertile agricultural land is often converted to residential, industrial, or infrastructural uses, reducing local production capacity and increasing reliance on extended supply networks (Cao & Wang, 2025).

Rising urban populations and higher incomes are driving dietary diversification in both quantity and quality. Demand is shifting toward higher-value foods like dairy, meat, fresh fruits, and processed convenience products, intensifying supply chain pressures for volume and authenticity (FAO, 2017). Globalization has created longer, more complex supply chains across countries with varying regulations, reducing transparency and complicating integrity monitoring. As production costs rise and profit margins shrink, economic pressures incentivize illicit cost-cutting practices, heightening food fraud risks.

Food fraud has existed for decades, evolving from simple adulteration to a sophisticated, multibillion-dollar criminal enterprise, with over 10% of global food supply affected annually (USP, 2021). Industry sources report cases increased nearly tenfold over the past four years (Eastlake, 2025), fueled by globalized markets, high-value commodities, just-in-time logistics, and regulatory differences enabling hard-to-detect practices (Yiannaka, 2023;

WTO, 2024). Prominent examples include the 2013 European horsemeat scandal, where 37% of 27 Irish beefburgers tested positive for horse DNA and 85% contained pig DNA (Department for Environment, Food & Rural Affairs, 2013), and the more recent 2021 Spanish saffron trafficking network, which sold €10M in fake premium saffron (OCCRP, 2021).

Fraud vulnerabilities are particularly high in premium commodities with significant economic value, such as spices, seafood, olive oil, honey, wine, and organic products, but this issue also affects staple foods like meat, grains, and dairy (Everstine et al., 2024; European Parliament, 2013; European Commission JRC, n.d.). Documented cases of fraud extend to other commodities, including edible oils, tea, coffee, beverages, dietary supplements, confectionery, alcoholic drinks, natural sweeteners, and food flavorings (Johnson, 2014). In 2024, seafood fraud represented 13% of all reported food fraud cases documented in FoodChain ID's Food Fraud Database, including species misrepresentation, the use of unapproved antibiotics and antifungal agents, and false claims regarding geographic origin (FoodChain ID, 2024). Similarly, honey fraud accounted for another 13%, typically involving the dilution of honey with exogenous sugar syrups (e.g., corn or rice syrup), the contamination arising from unapproved antibiotics used in bee colonies, and the marketing of honey-containing supplements adulterated with active pharmaceutical ingredients such as sildenafil and tadalafil as "natural" health products (FoodChain ID, 2024; Everstine et al., 2024; FDA, 2022).

In the dairy sector, fraud accounted for 12% of all reported food fraud cases (FoodChain ID, 2024). These acts included fraudulent labeling of products as sheep- or goat-milk-based when they contained cow's milk, the substitution of vegetable oils for milk fat, and the use of non-food-grade preservatives in milk. Moreover, herbs and spices remain a target for fraudulent activity, accounting for approximately 5.1% of all food fraud notifications. Of these, 25.6% were related to fraud and adulteration. Specific spices such as pepper (17%), cumin (14%), curcuma (11%), saffron (11%), and paprika/chili (6%) are particularly vulnerable to adulteration. Oregano, however, stands out as the most at-risk spice, with nearly 48% of samples contaminated, often with olive leaves (Everstine et al., 2024; FoodChain ID, 2024).

As fraud tactics evolve and diversify, the need for accurate, up-to-date, and comprehensive data on food fraud has never been more urgent. Companies must now examine more samples, navigate increasingly complex datasets, and rely on validated reference information to assess the authenticity of ingredients and products across a global supply chain. In response, platforms and databases such as FoodChain ID's Food Fraud Database (FoodChain ID, n.d.), the European Commission's Knowledge Centre for Food Fraud and Quality (European Commission JRC, n.d.), the Food Authenticity Network (Food Authenticity Network. (n.d.), and HorizonScan (HorizonScan, 2025) have become essential tools for consolidating global food fraud data. These systems are maintained by a combination of government agencies, research institutions, and commercial providers, and they draw data from regulatory alerts, scientific publications, media reports, laboratory

findings, and industry notifications. By aggregating this information, they allow organizations to identify emerging risks earlier and to adopt more proactive, intelligence-driven verification—meaning that companies can target checks, testing, and supplier reviews based on real-time signals of elevated fraud risk rather than relying solely on routine or generic controls (FoodChain ID, 2025; Spink et al., 2019; European Commission JRC, n.d.). However, even with improved access to food fraud data, weak or inconsistent traceability systems and differing regulations between countries still make it difficult to confidently verify product origin and quality (Trienekens, 2012; FAO, 2024).

The rise of e-commerce, especially over the last decade, has also significantly transformed food systems, making them increasingly "invisible" to consumers. This shift was accelerated by the COVID-19 pandemic, which drove unprecedented growth in online food purchasing and delivery as consumers avoided physical stores and relied more on digital channels for food (Reardon et al., 2023). Rapid logistics networks and dispersed supply chains have disrupted traditional food monitoring systems, leaving buyers more vulnerable to food fraud (Fernando et al., 2025). Online marketplaces, in particular, often lack rigorous mechanisms to verify authenticity or ensure compliance with national food safety standards (Vasileiou et al., 2025).

Delivery platforms like Uber Eats exemplify this through their reliance on dark or ghost kitchens—delivery-only facilities that provide ready-to-eat meals without dine-in spaces or visible storefronts (Hakim et al., 2020, 2023; da Cunha et al., 2024; Nigro et al., 2022). These operations cut costs by eliminating front-of-house staff and retail presence, passing savings to customers via lower prices (Khan, 2020; Kulshreshtha and Sharma, 2022). This shift brings a unique set of challenges and opportunities, particularly concerning food safety inspections and regulatory compliance. The anonymity of dark kitchens, coupled with their operational model, creates a higher risk for food fraud and safety violations, as their activities are less visible and harder to monitor (Laheri et al., 2025).

Amazon further exemplifies the challenges large platforms face in implementing food safety controls, as the scale of its third-party marketplace creates significant verification gaps despite requiring predictive analytics, vendor verification, and periodic audits. Even with mandatory onboarding checks and ongoing monitoring, the sheer number of sellers and product categories hinders full verification, highlighting how verification mechanisms on large online platforms may fall short in preventing food safety and authenticity risks (Lupo, 2021).

These structural challenges are not only operational but also regulatory. Policy frameworks, including the EU's Farm to Fork Strategy (European Commission, 2020) and the Codex Alimentarius guidelines (Codex Alimentarius Commission, n.d.), have emphasized the need for robust verification and traceability systems to safeguard food integrity in global markets. Analytical verification, documentation, and digital tracking systems often operate independently, limiting the ability to confirm product origin or detect fraud effectively. Overcoming these challenges requires shifting from reactive, end-point

monitoring to proactive, data-driven traceability frameworks that integrate analytical chemistry, digital tools, and advanced data analytics.

This thesis presents an integrated framework comprising three complementary systems: a physical traceability system, underpinned by advanced analytical techniques and database-driven authentication methods; a rapid traceability system, enabling fast and reliable spectroscopic screening for fraud detection; and a digital traceability system, which uses Internet of Things (IoT) technologies to provide real-time, continuous monitoring across the supply chain. Together, these systems integrate physical verification with digital transparency, enabling reliable authenticity assurance and verifiable supply chain monitoring.

1.1 Food Fraud and Traceability Challenges

Food fraud is a complex and multifaceted issue defined differently across regulatory and academic contexts, occurring at multiple points along the agri-food supply chain (Figure 1). From a regulatory standpoint, as outlined in Article 1(2) of Regulation (EU) 2017/625, "*food fraud encompasses any intentional infringement of agri-food chain legislation to deceive purchasers or consumers to obtain an undue economic advantage*". This regulatory definition emphasizes the deliberate nature of the act, the violation of food laws, and the intent to obtain an undue economic advantage by misleading purchasers or consumers.

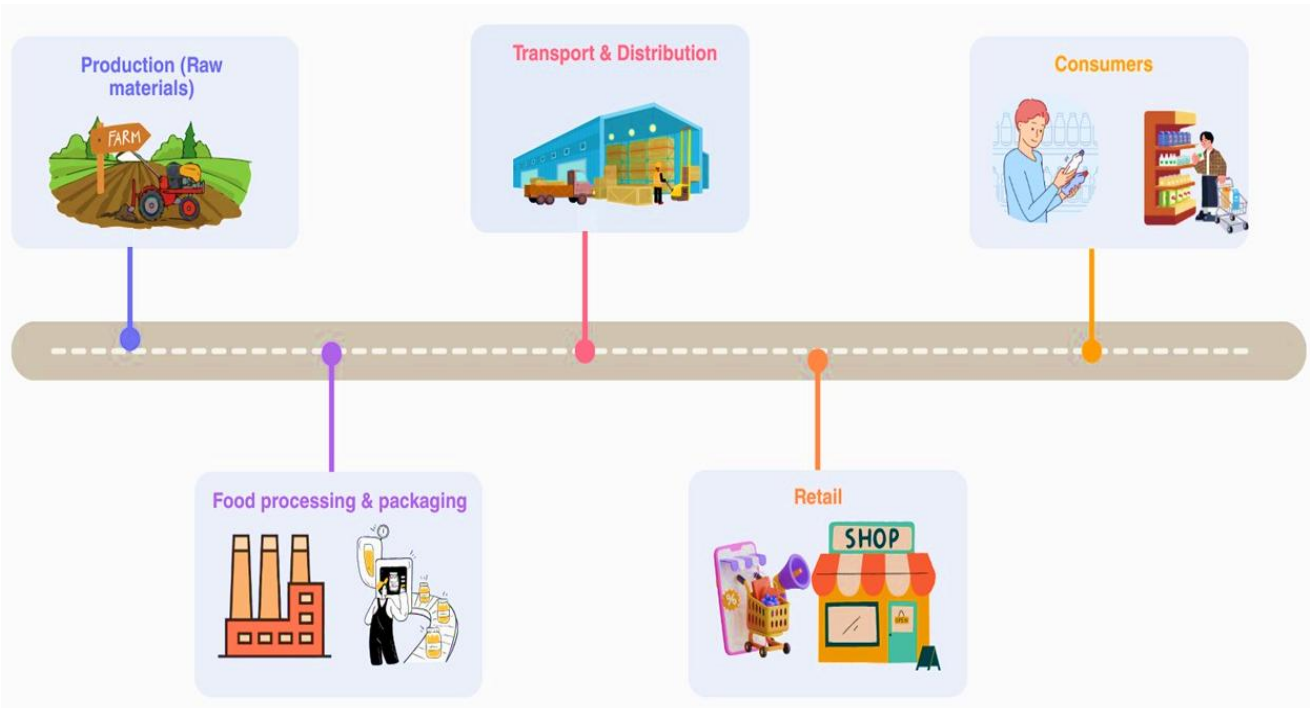


Figure 1: Food supply chain.

Complementing this regulatory framework, the widely cited academic definition by Spink and Moyer (2011) characterizes food fraud as "*a collective term for deliberate acts involving the substitution, addition, tampering, or misrepresentation of food, ingredients, or packaging, including false or misleading product information, all motivated by economic gain*". This broader view captures a range of deceptive practices which aim to provide financial benefit but can also pose risks to consumer health. By emphasizing the underlying human behavior—intentional deceit for economic profit—this definition highlights the diverse ways fraud can occur in food systems beyond legal definitions.

Spink and Moyer further categorize food fraud into seven primary types: (1) adulteration, (2) tampering, (3) over-run, (4) theft, (5) diversion, (6) simulation, and (7) counterfeiting (Table 1). These categories illustrate the varied mechanisms through which fraud undermines food authenticity (Spink & Moyer, 2011).

Table 1: Categories of food fraud and representative examples based on Spink & Moyer (2011).

Term	Definition	Examples
Adulteration	A component of the finished product is fraudulent	Mixing olive oil with cheaper oils like palm oil or adding melamine to milk.
Tampering and mislabeling	The genuine product and packaging are used fraudulently, including mislabeling the product's true content, character, origin, or utility.	Changed expiry information; fish labelled as "tuna" may actually be another type of fish.
Overrun	The legitimate product is made in excess of the production agreement	Under-reporting of production, where fraudulent products can be distributed outside of regulated or controlled supply chains.
Theft	The legitimate product is stolen and passed off as legitimately procured	Stolen products are merged with legitimate products.
Diversion	The sale or distribution of legitimate products outside of intended markets	Food aid is redirected to markets where aid is not required, leading to shortages or delays for needy populations.
Simulation	Dishonest products are designed to look like (but not identical to) the legitimate product.	Producing lesser-quality versions of popular foods, which are not produced with the same food safety and quality assurances.
Counterfeiting	All aspects of the fraudulent product and packaging are fully copied	Copying popular foods, but not produced with the same food safety and quality assurances.

Food fraud imposes substantial financial, reputational, and public health costs worldwide. The global economic impact on the food industry is estimated at USD 30–65 billion annually (Agetu, 2020; Bindt, 2016; Steinberg and Engert, 2019; RIKILT Wageningen UR, 2016). However, the true cost of food fraud is difficult to estimate, as discovering fraud is challenging, and the reported figures often underestimate broader consequences, such as diminished consumer trust, reduced competitiveness among legitimate producers, and increased regulatory costs. Cox et al. (2020) categorize these impacts into five dimensions: (1) victim costs, (2) criminal justice costs, (3) crime career costs, (4) intangible costs, and (5) market costs.

Victim costs include healthcare expenses and income loss when consumers suffer illness or injury, as evidenced by the 2008 Chinese melamine milk scandal. This incident resulted in nearly 300,000 infants falling ill and approximately 54,000 hospitalizations caused by milk products adulterated with toxic melamine, which had been added to artificially inflate apparent protein content (Gossner et al., 2009). Criminal justice costs involve law enforcement investigations and prosecutions, as demonstrated by recent European-wide operations targeting counterfeit and substandard food products. Such efforts, coordinated by agencies including Europol and OLAF, have resulted in the dismantling of multiple criminal networks, significant seizures of illicit goods, and numerous arrests (Europol, 2025). Crime career costs reflect the economic opportunities lost when individuals engage in fraudulent practices rather than legitimate business.

Intangible costs encompass declining consumer confidence and brand reputation, exemplified by scandals involving mislabeled “ethical” coffee brands (Cox et al., 2020; HorizonScan, 2025). Finally, market costs disadvantage honest producers, particularly those certified for organic or geographic authenticity, who lose market share to counterfeiters (Cox et al., 2020; FoodNavigator, 2025). As mentioned earlier, combating food fraud relies heavily on the concepts of authenticity and traceability, but what exactly do these terms mean?

Food authenticity focuses on verifying a product’s origin, composition, and production processes to ensure compliance with labeling and regulatory standards (CX/FICS 18/24/7, 2018). It guarantees that food products are genuine and that declared attributes are accurate, thereby preventing misrepresentation. Food traceability, closely linked to authenticity, involves tracking the movement of food through every stage of the supply chain. While definitions vary across frameworks, the core idea remains accountability. Regulation (EC) No 178/2002 describes traceability as "*the ability to trace and follow a food, feed, food-producing animal, or substance... through all stages of production, processing, and distribution.*" A logistics-based definition by Bosona and Gebresenbet (2013) emphasizes traceability as "*a part of logistics management that captures, stores, and transmits information at all stages in the food supply chain to ensure safety and quality control.*"

Traceability plays a dual role in both crisis management and fraud prevention, enabling rapid product recalls and strengthening consumer confidence. In practice, traceability systems document and link every step of the supply chain, allowing products to be traced backward to their origin and forward to their final destination (Golan et al., 2004). Beyond safeguarding public health by enabling rapid identification of contaminated products, traceability also helps combat counterfeiting, adulteration, and substitution. It further supports waste reduction and sustainability efforts by allowing companies to isolate only the affected items during incidents, thereby avoiding unnecessary disposal of large volumes of products (Spink & Moyer, 2011; FDA, 2022).

Transparency extends traceability by enabling the open and reliable sharing of information on a product's sourcing, production, and safety throughout the supply chain, thereby making traceability benefits visible to consumers (Trienekens et al., 2012). Increased transparency empowers consumers to make informed purchasing decisions, often favoring locally sourced or sustainably produced foods with lower carbon footprints (FAO, 2013). A 2018 survey by Label Insight and the Food Marketing Institute (FMI), involving 2,022 U.S. grocery shoppers, reported that 94% of consumers are more loyal to brands that provide transparent supply chain information, while 39% are willing to switch to brands demonstrating greater transparency (Label Insight, 2018). These expectations have accelerated the adoption of advanced technologies such as blockchain, which enable secure, real-time, and tamper-resistant data tracking across supply chains (Kamilaris et al., 2019). Companies that fail to meet transparency requirements increasingly risk losing consumer trust and competitiveness (Deloitte, 2020). As transparency and traceability rely on accurate and verifiable product information, robust analytical testing is essential for detecting adulteration and validating claims communicated along the supply chain.

In response to these evolving demands, governments have reinforced regulatory frameworks to enhance both food authenticity and traceability. In the European Union, the Official Controls Regulation (EU) 2017/625 mandates risk-based, unannounced inspections and coordinated cross-border enforcement to address food fraud more effectively. Competent authorities are empowered to conduct comprehensive controls at all stages of production and distribution, supported by the EU Food Fraud Network and the Administrative Assistance and Cooperation System, which facilitates rapid information exchange (European Commission, 2020). In the United States, Section 204 of the Food Safety Modernization Act (FSMA) requires stringent recordkeeping for high-risk foods to protect public health (FDA, 2022). Together, these legislative instruments promote standardized digital traceability practices, enabling quicker and more effective responses to contamination and fraudulent activities.

1.2 Approaches and Technologies for Strengthening Food Authenticity and Traceability

1.2.1 Analytical Testing Approaches for Food Authenticity

Advanced analytical techniques play an important role in maintaining food quality and consumer trust throughout the supply chain (Figure 2). However, these methods face well-recognized limitations, including long processing times, limited automation, and high operational costs (Manning & Soon, 2014; Ellis et al., 2012). Although combining methods such as elemental analysis, isotope-ratio mass spectrometry (IRMS), and complementary spectroscopic techniques does not eliminate these operational constraints, multimethod analytical strategies offer broader and more discriminative information that substantially strengthens authenticity verification (Camin et al., 2016; Reid et al., 2006), as demonstrated in the case studies presented in this dissertation. A summary of key analytical techniques, together with their main advantages and limitations, is presented in Table 2.

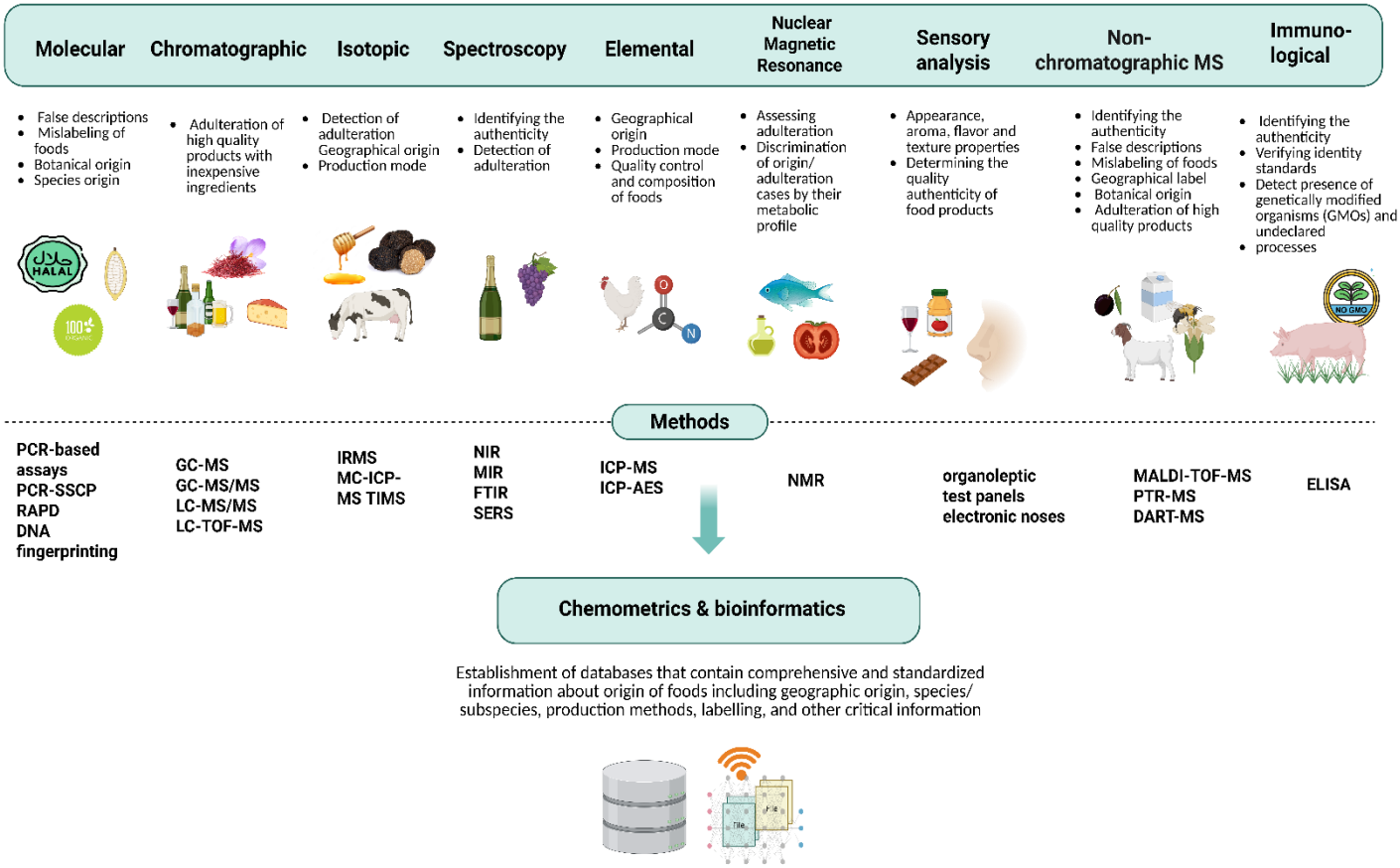


Figure 2: Overview of analytical methods for food authenticity and traceability.

Molecular techniques: These include genomics and proteomics which are widely used in food analysis due to their accuracy, reliability, and sensitivity. DNA's high stability enables analysis of minimal sample amounts—sometimes as few as a few cells (Sun et al., 2014). These methods are commonly used to identify food mislabeling and fraudulent claims, such as detecting GMOs, verifying seafood authenticity, confirming halal and kosher meat, identifying horse meat and pork in beef, determining the botanical origin of products like olive oil, wine, tomato products, tea, and cocoa, and verifying the species origin of meat, milk, and fish (Danezis et al., 2016). In addition to detecting mislabeling and false claims, these methods are also used to verify production methods (e.g., wild vs. farmed), technological processes, and trace contaminants, ensuring the traceability of highly processed food products (Danezis et al., 2016; Su et al., 2024). Standard genomic and proteogenomic authentication techniques include polymerase chain reaction (PCR) and its variants, such as PCR–single-strand conformation polymorphism (PCR-SSCP), random amplified polymorphic DNA (RAPD), DNA fingerprinting, and peptide nucleic acid (PNA)-based assays (Sforza et al., 2011).

Chromatographic techniques: Chromatographic analysis offers rapid and reliable separation of chemically similar compounds in complex food matrices, overcoming challenges posed by the diversity of compounds in food substrates, such as peptides, lipids, carbohydrates, and small molecules like colorants, aromas, and preservatives (Cserháti et al., 2005). These compounds vary in size, from small organic molecules (typically up to 1000 Da) to macromolecules (biopolymers), and exhibit a wide range of polarities. Chromatographic methods, particularly those coupled with mass spectrometry (MS), such as gas chromatography–mass spectrometry (GC–MS/MS), liquid chromatography–mass spectrometry (LC–MS/MS), and liquid chromatography–time-of-flight mass spectrometry (LC–TOF-MS), generate unique chemical fingerprints that are crucial for food authentication. These methods are widely used to detect proteins, amino acids, carbohydrates, vitamins, triglycerides, chiral compounds, and pigments, with gas chromatography better suited for volatile compounds (Ibáñez et al., 2013). Authentication by chromatography relies on comparing compound profiles, such as fatty acids, triglycerides, sterols, and volatiles, to those characteristics of specific food origins. Applications include detecting adulteration in high-quality products such as honey, wine, oils, coffee, milk, saffron, and nuts by matching measured profiles with predetermined reference values (Di Stefano et al., 2012).

Spectroscopic techniques: Such methods are valued for their speed, non-destructive nature, and cost-effectiveness. Vibrational and infrared techniques, such as near-infrared (NIR), mid-infrared (MIR), FTIR, and surface-enhanced Raman spectroscopy (SERS), provide information on the chemical and physical characteristics of samples (Hayden, 2001; Cozzolino, 2012). Fluorescence spectroscopy, particularly total synchronous fluorescence (TSyF), offers very low detection limits (ng/mL to pg/mL) and has been widely used to evaluate authenticity, adulteration, quality, and composition (Poulli et al., 2007). Nuclear magnetic resonance (NMR) spectroscopy, including site-specific natural isotopic

fractionation (SNIF-NMR), further enhances authentication by providing high-resolution metabolic profiles of foodstuffs. Because NMR can characterize a wide range of molecular compounds such as amino acids, sugars, organic acids, and fatty acids, it enables detailed compositional fingerprinting across many product types. SNIF-NMR has long been used in the EU for verifying the geographical origin of wine (Godelmann et al., 2013; Solovyev et al., 2021), while non-targeted ^{1}H-NMR profiling has been successfully applied to discriminate origin and detect adulteration in products such as olive oil, wine, coffee, honey, maple syrup, fish, spirits, vinegar, and saffron (Alonso-Salces et al., 2015; Longobardi et al., 2013; Ogrinc et al., 2003). Continuous advances in hardware have also significantly improved NMR sensitivity, expanding its applicability to routine food authenticity testing (Danezis et al., 2016).

Elemental profiling: A method valued for its ability to reveal the elemental composition of foods, ranging from macro-elements to trace and rare earth elements. It is based on the principle that plants absorb elements from the soil, and animals acquire them through water, food, and vegetation. Factors such as soil type, fertilization, pollution, botanical origin, and production year influence these elemental concentrations, making elemental fingerprints useful for determining provenance and detecting adulteration (Drivelos et al., 2012; Reinholds et al., 2015; Laursen et al., 2014). The most widely used techniques are Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES), known for their capacity to perform multi-element measurements. These methods are applied in a variety of contexts, including distinguishing between free-range and cage-produced eggs, as well as identifying the origins of products such as wine, honey, olive oil, coffee, cheese, fruits, vegetables, spices, and food additives (Drivelos et al., 2012; Laursen et al., 2014).

Isotopic profiling: This approach examines the natural variations in the ratios of light (^{2}H/^{1}H, ^{13}C/^{12}C, ^{15}N/^{14}N, ^{18}O/^{16}O, ^{34}S/^{32}S) and heavy (^{87}Sr/^{86}Sr, ^{206}Pb/^{204}Pb, ^{207}Pb/^{204}Pb, ^{208}Pb/^{204}Pb, ^{143}Nd/^{144}Nd) isotopes within food matrices (Kelly et al., 2005). These isotopic ratios reflect environmental and geological conditions such as climate, soil composition, altitude, and agricultural practices at the site of production, making them powerful indicators of geographical origin and authenticity (Drivelos et al., 2012). The most commonly applied analytical methods include Isotope Ratio Mass Spectrometry (IRMS) for light isotopes and Multi-Collector Inductively Coupled Plasma Mass Spectrometry (MC-ICP-MS) or Thermal Ionization Mass Spectrometry (TIMS) for heavy isotopes.

Complementary approaches also extend to sensory analysis, where organoleptic panels and electronic sensing systems—such as e-noses, e-tongues, and e-eyes—are used to assess appearance, aroma, flavor, and texture markers relevant to food authenticity. These methods are rapid, low-cost, and require minimal sample preparation (Elortondo et al., 2007; Strike et al., 1999). Similarly, non-chromatographic mass spectrometry methods, such as Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF-MS), Proton Transfer Reaction Mass Spectrometry (PTR-MS), and ambient mass spectrometry techniques (e.g., Direct Analysis in Real Time Mass

Spectrometry [DART-MS]), have been employed to detect fraudulent claims, mislabeling, adulteration, and the botanical origin of spices and other products (Luykx & Van Ruth, 2008).

Immunological assays provide another widely applied category. Based on antibody–antigen interactions, these techniques are fast, sensitive, and highly specific (Nielsen, 2010). Enzyme-linked immunosorbent assay (ELISA) is the most common format and has been successfully applied to the authentication of meat, fish, and dairy products (Kim et al., 2023; Chen & Hsieh, 2014; Eldahshoury & Hurley, 2023) as well as to the detection of GMOs and undeclared processing, such as food irradiation (Asensio et al., 2008).

Table 2: Analytical techniques for food authenticity and traceability: methods, advantages, and limitations (Danezis et al., 2016).

Method	Advantages	Disadvantages
Molecular Techniques (Genomics & Proteomics)	High accuracy, reliability, minimal sample required	High cost, complex equipment, and time-consuming for large sample sizes
Chromatographic Techniques	High resolution, reliable separation	Requires sophisticated equipment, is time-consuming, and expensive
Spectroscopic Methods (NIR, MIR, FTIR)	Rapid, non-destructive, cost-effective, minimal sample prep, simultaneous multi-component analysis, high-throughput screening	Sensitivity limitations for trace compounds, water interference, especially in IR, need for robust calibration and expertise
Sensory Analysis	Low-cost, simple, rapid	Subjective, needs trained panelists
Non-Chromatographic Mass Spectrometry	Very rapid analysis, minimal sample preparation, high sensitivity, suitable for complex matrices	Limited compound separation, potential matrix effects, and high instrument cost
Immunological Assays (ELISA)	Fast, sensitive, easy to use	Requires antibodies, limited application range
Isotope Ratio Mass Spectrometry (IRMS)	High-precision isotopic ratio data is the gold standard for provenance and authenticity	Expensive instrumentation, complex operation, longer analysis time
Elemental Analysis	Provides elemental composition, useful for authenticity and quality control	Requires specialized equipment, moderate cost, and sample prep

1.2.1.1 Stable Isotope, Elemental, and FTIR Analyses for Geographic Traceability and Production Process Characterization

While analytical methods are powerful tools for determining product authenticity—verifying its composition, origin, or quality—not all of these techniques are suitable for traceability purposes. Analytical authentication approaches such as spectroscopy and chromatography provide a snapshot of a product's intrinsic properties at a specific point in time. However, they do not inherently capture the movement or transformation of goods along the supply chain. However, specific analytical techniques can enhance traceability by providing provenance markers that persist throughout the production and distribution stages. Among these, stable isotope ratio analysis (SIRA) and elemental fingerprinting are considered the most reliable and robust methods (Kelly et al., 2005; Katerinopoulou et al., 2020), performed using an IRMS equipped with a preparative module for solid samples—such as the IsoPrime100 coupled to a Vario PYRO Cube —and a triple-quadrupole ICP-MS (ICP-QQQ), respectively.

1.2.1.1.1 Isotopic Fractionation and Its Role in Food Authentication

The high precision of stable isotope methods provides clear and robust evidence for food authentication, with routine IRMS measurements typically achieving repeatability on the order of a few tenths of a per mil for $\delta^{13}C$ and $\delta^{15}N$ and around 1–3‰ for $\delta^{2}H$ in food matrices—performance generally not attainable with many other analytical techniques

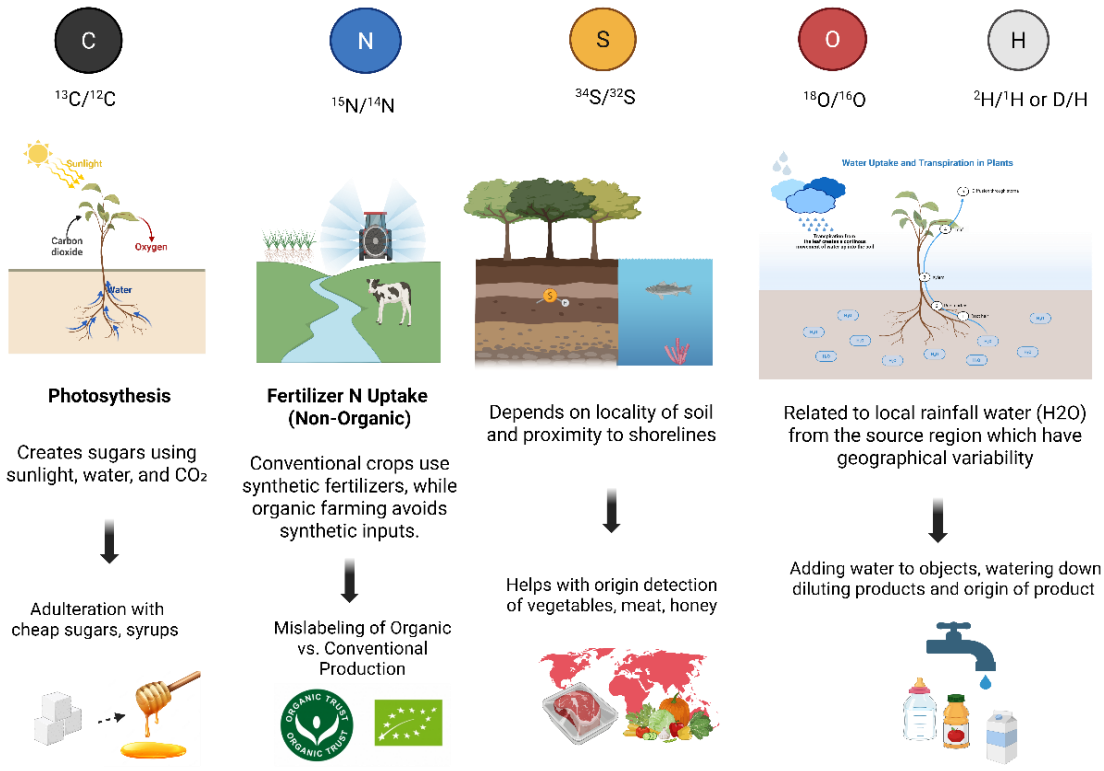


Figure 3: Stable isotopes and their presentation in food origin and authenticity.

(Camin et al., 2016; Kelly et al., 2005; Werner & Brand, 2001). This technique is also efficient, requiring only small amounts of homogenized sample to produce accurate results and making it suitable for routine use even when sample availability is limited (Kelly et al., 2005). Moreover, stable isotope approaches can be applied to a wide range of commodities and production systems, supporting detection of mislabeling and fraud across global food supply chains (Camin et al., 2017; Laursen et al., 2016). Beyond food authentication, the distinctive properties of stable isotopes have enabled applications in environmental monitoring, water and soil management, nutrition studies, and forensic science (IAEA, 2016).

Stable isotope analysis is grounded in natural isotopic fractionation processes. Fractionation arises because physical, chemical, and enzymatic reactions discriminate between heavy and light isotopes, producing systematic differences in isotope ratios among environmental reservoirs and biological tissues (Drivelos et al., 2012; Farquhar et al., 1989; Gat, 1996). These differences encode information about climate, geology, soil chemistry, and biological metabolism, and the resulting isotopic patterns become embedded in food products. In food authentication, the most commonly used stable isotope ratios are $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$ and $^{34}S/^{32}S$ (Figure 3)[1]. Their values in food reflect (1) the isotopic signature of environmental inputs, (2) fractionation during physiological processes such as transpiration, photosynthesis, or nitrogen assimilation, and (3) influences of geography, climate, soil composition, botanical origin, and agricultural practices (Camin et al., 2016).

Hydrogen and oxygen isotopes ($\delta^{2}H$ and $\delta^{18}O$) in plant tissues originate from local precipitation and soil water, which vary systematically with latitude, altitude, continentality, and evapotranspiration (Gat, 1996; Welp et al., 2006). Globally, $\delta^{2}H$ in precipitation typically ranges from –20‰ to –60‰ in coastal low-latitude regions, –50‰ to –120‰ in temperate continental areas, and –120‰ to –200‰ in high-latitude or high-altitude environments (Bowen & Revenaugh, 2003; Gat, 1996). $\delta^{18}O$ follows similar spatial gradients, with values of 0‰ to –4‰ in coastal low-latitude climates, –4‰ to –12‰ in temperate continental regions, and –12‰ to –20‰ in high-altitude or high-latitude areas (Dansgaard, 1964; Gat, 1996). Root water uptake does not fractionate, while leaf water undergoes evaporative enrichment, transferring these environmental signals into plant organic matter—typically enriching cellulose by +5‰ to +20‰ for $\delta^{18}O$ and +20‰ to +80‰ for $\delta^{2}H$ relative to source water (Roden & Ehleringer, 2000; Yakir & DeNiro, 1990). These isotopic signatures subsequently pass into animal tissues through diet and drinking water (Sternberg, 1989).

[1] Measurements of the stable isotope ratios of light elements are expressed in the δ-notation in ‰ according to the equation: $\delta^{i}E = (R(^{i}E/^{j}E)_{sample} / R(^{i}E/^{j}E)_{standard}) - 1$, where i stands for the highest and j for the lowest atomic mass number of the element E (C, N, O, S), and R is the isotope ratio between the heavier and the lighter isotope of the element ($^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{34}S/^{32}S$) in the *sample* or *standard*. The $\delta^{13}C$ values are expressed relative to V-PDB (Vienna-Pee Dee Belemnite) standard, $\delta^{15}N$ values relative to AIR, $\delta^{34}S$ values relative to V-CDT (Vienna-Canyon Diablo Troilite) standard and the $\delta^{2}H$ and $\delta^{18}O$ values relative to the VSMOW (Vienna-Standard Mean Ocean Water) standard.

Carbon isotopic ratios ($\delta^{13}C$) serve as robust tracers of photosynthetic pathways in plants. C_3 plants—such as wheat, rice, barley, soybeans, potatoes, spinach, and most trees—typically exhibit $\delta^{13}C$ values between –23‰ and –34‰ (mean ≈ –27‰), reflecting strong discrimination against $^{13}CO_2$ by Rubisco during CO_2 fixation (Farquhar et al., 1989; O'Leary, 1988). In contrast, C_4 plants—like maize (corn), sugarcane, sorghum, millet, and tropical grasses—range from –9‰ to –16‰ (mean ≈ –13‰) due to initial fixation by phosphoenolpyruvate (PEP) carboxylase, which shows minimal fractionation.

Nitrogen isotopes ($\delta^{15}N$) are influenced by soil nitrogen sources, fertilizer use, climate, and land management, making them useful for distinguishing between organic and conventional farming as well as geographic origins (Camin et al., 2016; Gatzert et al., 2021). Nitrogen isotopes ($\delta^{15}N$) reflect the isotopic composition of soil nitrogen pools and fertilizers. Synthetic fertilizers generally show $\delta^{15}N$ values from –4‰ to +4‰, whereas manure- or compost-derived nitrogen typically ranges from +8‰ to +20‰ because of ammonium volatilization and microbial transformations (Bateman & Kelly, 2007). These values are transmitted into plant amino acids during assimilation and subsequently into animal tissues with a trophic enrichment of approximately +3–5‰.

$\delta^{34}S$ values reflect geological and anthropogenic factors, including lithology, marine sulfate deposition, volcanic inputs, and industrial emissions (Danezis et al., 2016; Gatzert et al., 2021). Marine-derived sulfate typically exhibits $\delta^{34}S$ values of +17‰ to +21‰, coastal soils influenced by marine aerosols range from +10‰ to +20‰, terrestrial sulfate sources generally fall between 0‰ and +10‰, and reduced, or volcanic sulfur can be highly depleted, ranging from –20‰ to 0‰ (Krouse & Grinenko, 1991; Trust & Fry, 1992). Plants absorb sulfate from soil with negligible fractionation, and the isotopic composition is retained during reduction and incorporation into sulfur-containing amino acids. Consequently, $\delta^{34}S$ values in plant tissues typically fall within the range -5‰ to +20‰, making sulfur isotopes a sensitive marker of geological and anthropogenic influences.

Because these isotope ratios integrate environmental and agricultural conditions, they have demonstrated strong discriminatory power in food authentication. Several studies have successfully used stable isotopes to differentiate production systems (e.g., free-range vs. grain-fed yak meat; Yang et al., 2023), farming practices (organic vs. conventional milk; Molkentin & Giesemann, 2007), and the geographical origin of animal-derived products including pork (Dehelean et al., 2023), beef (Zhao et al., 2013), poultry (Zhaxi et al., 2021), milk (Potočnik et al., 2020), butter (Rossmann et al., 2000), cheese (Manca et al., 2006), and seafood (Carrera et al., 2017).

1.2.1.1.2 Combining Stable Isotopes with Elemental Analysis and FTIR for Robust Verification

When combined with elemental composition analysis, the verification of food authenticity becomes even more robust. The availability and uptake of elements depend on multiple environmental and geological factors. Soil characteristics such as pH, moisture, and clay content, as well as climate and water availability, play a critical role (Katerinopoulou et al., 2020). Elements including Sr, Ba, Cs, S, Mo, and Ni are strongly

associated with local geology (Saaltink et al., 2014). For instance, strontium isotopes are already widely used to trace the geographical origin of agricultural products (Gregorčič et al., 2021; Hiraoka et al., 2016), while rubidium and cesium, which are readily mobilized in soil, can serve as useful markers of plant uptake (Kelly et al., 2005). Sodium content is also influenced by proximity to the sea, with higher concentrations typically observed within a few kilometers of the coast due to marine aerosol deposition (Saaltink et al., 2014).

Agricultural practices also influence the levels of many elements (Martínez-Ballesta et al., 2010). Phosphorus, for example, is closely linked to the use of phosphoric fertilizers and other agrochemicals (e.g., glyphosate-based herbicides and organophosphate insecticides). Although earlier research suggested minimal impact on elemental profiles for origin tracing (Smith, 2005), this is contradicted by more recent studies. For instance, fertilizer treatments on highland barley in Tibet significantly altered mineral element levels, including P, K, Fe, and Cu, thereby improving geographic classification accuracy (Zhao et al., 2022). Similarly, elevated concentrations of toxic elements such as As, Cd, and Cu may result from intensive fertilizer and pesticide use, particularly in heavily farmed areas (Skordas et al., 2013). These findings underscore the need for careful selection of elemental markers, since they may reflect anthropogenic influences rather than natural environmental signatures.

Complementary to stable isotope and elemental analyses, Fourier Transform Infrared (FTIR) spectroscopy offers a rapid, non-destructive, and cost-effective analytical approach for food authenticity and traceability. Unlike isotopic and elemental analyses, FTIR captures information about molecular composition and chemical functional groups, enabling the differentiation of food products based on their biochemical profiles (Ellis et al., 2012; Rohman, 2020; Rodriguez-Saona, 2016). FTIR spectra are, therefore, highly sensitive to compositional changes arising from geographical, climatic, soil, botanical, and processing factors. For example, FTIR has been successfully used to authenticate the geographical origin of honey, olive oil, milk, and saffron, as well as to detect adulteration in spices and dairy products (Mendes & Duarte, 2021; Ahmad & Ayub, 2022; Valand et al., 2020). Absorption bands associated with key functional groups (e.g., C–H, O–H, N–H, and C=O) provide diagnostic spectral information that helps differentiate authentic samples from adulterated ones. For instance, C–H stretching vibrations are characteristic of lipid-rich organic compounds such as oils and fats, whereas O–H bands are commonly used to assess water content in beverages like milk (Ellis et al., 2012). As a practical example, FTIR spectroscopy using normal spectra in the 3098–669 cm^{-1} region has been successfully applied to distinguish pure bovine milk fat from samples adulterated with lard, with clear separation achieved even at adulteration levels as low as 5% (w/w) (Windarsih et al., 2020).

The advantage of FTIR lies in its speed and simplicity: minimal sample preparation is required, data acquisition typically takes only a few seconds, and the sample undergoes negligible physical or chemical alteration during measurement. This makes FTIR particularly suitable for high-throughput screening and in-field analysis, especially when

combined with portable or handheld instruments integrated with cloud-based data systems (Ellis et al., 2012). Moreover, FTIR produces minimal waste and requires no chemical reagents, aligning with green analytical chemistry principles (Nasr et al., 2020; Cherniienko et al., 2024).

Despite its practical advantages, FTIR spectroscopy faces inherent physical and chemical limitations when used as a stand-alone tool for complex food matrices, especially compared with elemental- and stable isotope-based methods. A primary challenge is the extensive spectral overlap of absorption bands: in multi-ingredient food systems, major organic compounds (proteins, lipids, and carbohydrates) generate overlapping vibrational signals, and high water content produces dominant O–H stretching and bending vibrations, both of which can mask subtle differences or obscure low-abundance analytes required for precise geographic or compositional tracking (Rodriguez-Saona & Allendorf, 2011; Valand et al., 2020; Lohumi et al., 2015).

Physical matrix properties—such as light scattering from fat emulsions or crystalline particulates—can further distort optical paths and introduce baseline drift (Valand et al., 2020). While elemental methods (e.g., ICP-MS) bypass matrix interference via destructive acid digestion and isotope methods (e.g., IRMS) provide highly specific atomic and isotopic measurements (Mazarakioti et al., 2022; Camin, 2016), FTIR heavily depends on sample uniformity. Consequently, spectroscopic approaches excel at rapid screening but are generally limited to percent (%) or parts-per-million (ppm) detection levels, making ultra-trace marker identification challenging without advanced chemometric preprocessing (Rodriguez-Saona & Allendorf, 2011; Valand et al., 2020). Consequently, integrating these methods provides a powerful analytical synergy: elemental and isotopic mass spectrometry workflows establish a legally robust geographical provenance, while FTIR serves as a rapid, non-destructive screen for immediate molecular composition and fraud detection (Valand et al., 2020)

FTIR's full potential is realized when integrated with chemometric and machine learning algorithms. Techniques like Principal Component Analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA), and Support Vector Machines (SVM) enhance the processing of complex spectral data, allowing for accurate classification and detection of subtle compositional differences. For example, FTIR coupled with PCA has been shown to distinguish saffron samples according to their regional origin and detect dilution with lower-grade materials, while PLS regression models have been applied to quantify compositional parameters such as moisture or fat content in dairy and meat products (Santos, 2013; Petrakis, 2017; Li, 2019). Furthermore, FTIR combined with machine learning algorithms has been successfully used to classify Mediterranean honey by geographical and botanical origin (Dimakopoulou-Papazoglou et al., 2025), and to detect adulteration in fruit juices with algorithms like SVM, Random Forest, and Linear Discriminant Analysis, demonstrating FTIR's robust applicability for food authenticity across both solid and liquid matrices (Calle et al., 2022).

1.2.2 Databases

The effectiveness of analytical techniques depends on well-curated, standardized reference databases that contextualize isotopic and elemental signatures, enabling reliable comparisons across regions, seasons, crop varieties, and processing methods. Without such frameworks, even high-resolution data may remain ambiguous or inconclusive (Camin et al., 2016). Repositories like IsoBank (Shipley et al., 2024) and StabisoDB (Grossman et al., 2022) offer valuable stable isotope data but are limited by commodity coverage, inconsistent metadata, and a lack of integration with food-specific variables. Additionally, many datasets are siloed within national or institutional boundaries, hindering interoperability for broader scientific and regulatory use.

A comprehensive food authenticity database must be built on established protocols and populated with representative authentic samples that capture natural variability (Donarski et al., 2019). This variability serves as a crucial reference for identifying fraud, including mislabeling and adulteration. To be effective, such databases must cover a wide range of authentic samples representing geographic, seasonal, dietary, and production differences (Camin et al., 2016). Commercial samples can then be compared against this reference material within defined statistical limits. However, creating and maintaining these resources requires significant investment, long-term funding, and rigorous curation (Kelly, 2019). Ideally, samples should be collected directly from primary producers to ensure integrity and traceability (Donarski et al., 2019; Di Egidio et al., 2011). Particular attention is needed for high-variability commodities such as fruits and vegetables, which require yearly updates (Camin et al., 2017).

Equally important is the standardization and harmonization of metadata and analytical results to ensure reproducibility and comparability across laboratories (Berg et al., 2013). Metadata should include critical contextual information such as sample origin, harvest date, production method, cultivar, processing steps, and analytical conditions. To achieve consistency and interoperability, structured vocabularies and ontologies provide a framework for semantic integration of data. By enabling machine-readable metadata and cross-platform linking, ontologies ensure that datasets are standardized, extensible, and aligned with FAIR principles (Findable, Accessible, Interoperable, Reusable).

To address existing gaps in food authenticity and traceability, the IsoFoodTrack database was recently developed as a scalable, flexible platform to organize open-access stable-isotope, elemental, and related data across a broad range of food commodities (Terro et al., 2025). The ISOFOOD ontology (Eftimov et al., 2019) complements the database by providing a semantic framework that standardizes the description and categorization of isotopic and elemental data. This ontology ensures that metadata used in IsoFoodTrack follows a consistent protocol, thereby improving data quality and supporting cross-study comparisons. The ISOFOOD ontology also enhances interoperability by enabling the

integration of IsoFoodTrack with other open-access databases, facilitating a more holistic approach to food authenticity and traceability research (Figure 4).

While these technical frameworks improve data quality and interoperability, realizing the full potential of such databases requires critically addressing questions of long-term governance and sustainability. As contributions from diverse laboratories accumulate, systematic differences in instrument calibration, sample preparation protocols, and reference material selection risk introducing inter-laboratory variability that could undermine the reliability of cross-study comparisons (Berg et al., 2013). Establishing formal governance structures — including defined data ownership, tiered access rights, and mandatory quality control protocols — will therefore be essential to ensure the integrity and long-term utility of the platform (Donarski et al., 2019). Furthermore, institutional data ownership policies, intellectual property concerns, and competitive interests among industry stakeholders represent substantive barriers to data sharing that technical solutions alone cannot overcome (Kelly, 2019). Addressing these issues will require coordinated policy-level commitments to open science, alongside inter-laboratory validation exercises and standardized operating procedures that ensure consistency and trust across contributing institutions (Berg et al., 2013; Kelly, 2019).

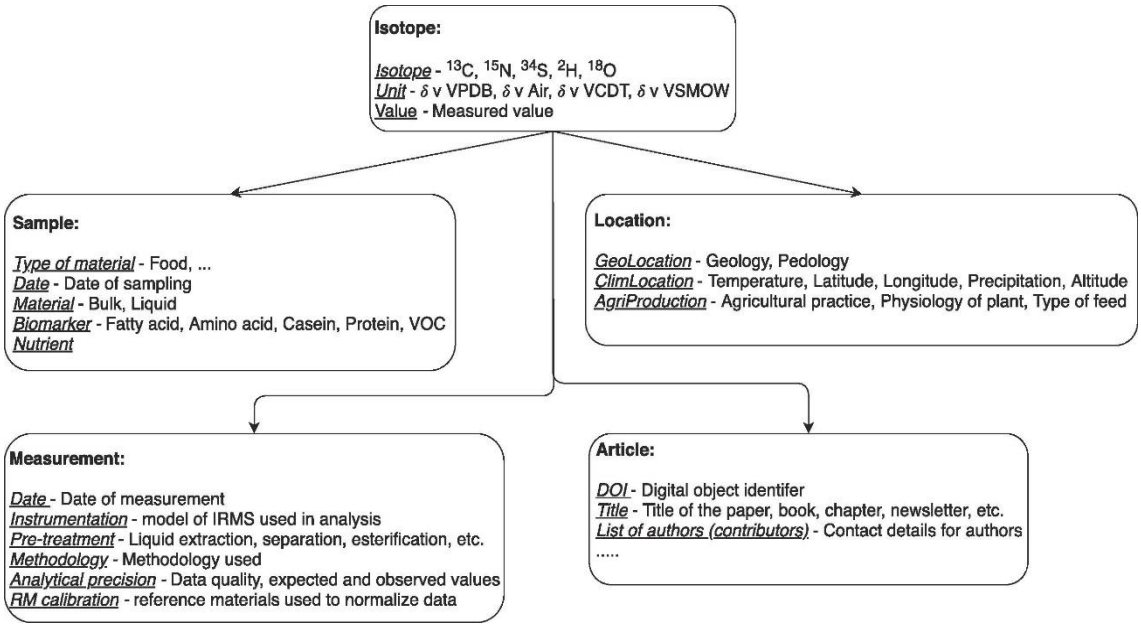


Figure 4: Metadata and provenance data of the proposed ISO-FOOD ontology (adopted by Eftimov et al., 2019).

Once the samples are acquired, the data need to be analyzed using multivariate statistical models, such as discriminant analysis, MANOVA, and class modeling, to classify

products by origin, species, or production method. These statistical models are essential for distinguishing authentic food products from fraudulent ones.

1.2.3 Data Analysis Approaches: Chemometrics and Artificial Intelligence

Chemometric techniques, also known as multivariate data analysis methods, are crucial for uncovering meaningful patterns in complex datasets by separating noise from relevant signals and identifying hidden relationships. The three main chemometric strategies, exploratory analysis, classification, and class modeling, are widely used in food science, with the selected method tailored to the specific research objectives and data characteristics (Granato et al., 2018).

Principal Component Analysis (PCA) is frequently used as an initial step to reduce dimensionality and extract features. As an unsupervised technique, PCA generates new composite variables, called principal components, that capture the most informative variance while minimizing information loss (Covaciu et al., 2016). Because PCA does not utilize group labels, supervised classification methods are preferred when the goal is to distinguish samples by origin or category. These classification approaches, often termed discriminant analyses, assign samples to predefined groups and identify the variables that most effectively discriminate between them (Marini, 2009). Typical classifiers include Linear Discriminant Analysis (LDA), k-Nearest Neighbors (k-NN), Partial Least Squares Discriminant Analysis (PLS-DA), and Artificial Neural Networks (ANN).

LDA is a simple classifier but requires an adequate sample-to-variable ratio and can struggle with highly collinear chemical datasets. To address such challenges, PLS-DA and its variant Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) were developed, with the latter improving model interpretability by separating predictive variance from non-predictive (orthogonal) variance (Rongai et al., 2017). Model fitness is assessed with explained variance (R^2) and predictive ability (Q^2), using internal cross-validation to reduce overfitting risk. Classification accuracy is evaluated through sensitivity and specificity, while influential variables are detected using Variable Importance in Projection (VIP) scores (Fiamegos et al., 2021). Several studies using stable isotope and elemental data have confirmed the utility of discriminant techniques for identifying the geographical origins of food products (e.g., Bat et al., 2012; Fiamegos et al., 2021). However, in one-class classification scenarios, where the target class is well defined but the non-target class is underrepresented or highly diverse, these methods may yield unreliable results (Oliveri, 2017).

Traditional discriminant analysis techniques, though widely used, are inherently limited because they require all samples to belong to predefined classes. This assumption prevents them from identifying unknown or novel samples that may arise in real-world authentication scenarios. To overcome this limitation, class-modeling or one-class classification methods, such as Soft Independent Modeling of Class Analogy (SIMCA), are becoming more widely recommended for authentication and verification tasks (Oliveri,

2017; Rodionova et al., 2016). This approach has been successfully implemented across a range of food matrices, including fruits and vegetables (Mahynski et al., 2025), strawberries (Strojnik et al., 2022), fish (de Andrade et al., 2024; Machado et al., 2025), instant coffee (Candeias et al., 2025), tea (Fechner et al., 2024), and sweeteners (de Sousa et al., 2025).

This thesis adopts the data-driven SIMCA (DD-SIMCA) variant, which enhances the classical SIMCA framework by incorporating a statistically rigorous, data-driven methodology (Pomerantsev & Rodionova, 2014). In DD-SIMCA, PCA is performed on training data to define an acceptance region that characterizes the target class. Two fundamental parameters describe each sample's relationship to this region: the score distance (SD), representing its position within the principal component space, and the orthogonal distance (OD), which quantifies deviation outside this modeled space. These distances, treated as population statistics, are combined to determine a total distance that defines the classification threshold for the target class. The resulting acceptance plot (Zontov et al., 2017) provides an intuitive visual representation of the model, where the proximity of a sample to the modeled class reflects its degree of similarity—samples closer to the class center are more alike, while those farther away show greater differentiation (de Andrade et al., 2024; Strojnik et al., 2022).

One of DD-SIMCA's key advantages is its probabilistic approach to classification. A test sample is assigned to the target class only if it satisfies the model's acceptance criteria with a sufficiently high degree of probability. If the residual variance of a sample exceeds the upper confidence limit for all modeled classes, it remains unclassified—indicating that it may be an outlier or belong to an unrepresented class within the dataset (Pirhadi et al., 2015). This makes DD-SIMCA particularly effective for identifying adulteration, detecting atypical samples, or verifying geographical origin.

Model performance in DD-SIMCA is evaluated using sensitivity and specificity, corresponding to the correct identification of target-class samples and the correct exclusion of non-target samples, respectively. These metrics parallel the concepts of type I and type II errors in statistical testing (Fidelis et al., 2017). Owing to its robust one-class classification framework and the ability to estimate misclassification probabilities theoretically, DD-SIMCA offers a highly reliable and interpretable tool for food authenticity assessment and origin verification.

Further, to enhance classification accuracy and predictive performance beyond chemometric methods, this study integrates artificial intelligence (AI), particularly machine learning, into the analytical workflow. Machine learning, a subset of AI, leverages algorithms capable of learning complex patterns in high-dimensional stable isotope and elemental data, thereby improving model accuracy and robustness (Gbashi & Njobeh, 2024). However, AI models—especially complex ones—often function as "black boxes," limiting interpretability and user trust.

To address this, Explainable Artificial Intelligence (XAI) approaches can be used to provide transparent, interpretable outputs that clarify the reasoning behind AI decisions (Doshi-Velez & Kim, 2017; Gunning, 2017). Methods such as SHAP (SHapley Additive exPlanations), LIME (Local Interpretable Model-agnostic Explanations), and Grad-CAM (Gradient-weighted Class Activation Mapping) offer detailed insights into which features most influence classification outcomes, enhancing stakeholders' confidence in AI models (Buyuktepe et al., 2025). By revealing the underlying model logic, XAI not only improves understanding of feature importance but also supports reliable authentication (Arrieta et al., 2020).

Combining the predictive power of AI with XAI's interpretability, this integrated approach enhances classification performance while fostering trust and practical applicability, such as verifying the geographic origin of food products (Guidotti et al., 2018; Samek et al., 2017). Beyond laboratory applications, AI and ML extend these benefits to the supply chain, enabling the analysis of large datasets from traceability systems—including blockchain, IoT, and RFID—to detect anomalies, predict fraud risks, and guide proactive interventions (Gbashi & Njobeh, 2024).

1.2.4 Applications and Case Studies on Selected Spices

The combination of stable isotope and elemental analysis has been successfully applied to a variety of high-value food products, such as honey (Wu et al., 2015; Bontempo et al., 2017), olive oil (Camin et al., 2010), sweet pepper (Opatić et al., 2017), paprika (Brunner et al., 2010; Primožič et al., 2025), saffron (Wakefield et al., 2019; Perini et al., 2020), coffee beans (Santato et al., 2012), apple juice (Bizjak Bat et al., 2016), strawberries (Strojnik et al., 2022), wine (Leder et al., 2021) seafood and shellfish (Ni et al., 2022; Luo et al., 2019), beef (Bai et al., 2025), and lamb (Liu et al., 2020). In addition, it has been applied to rice, garlic, and Chinese medicinal materials (Giannioti et al., 2024; Opatić et al., 2017; Zhang et al., 2022). This integrated approach is particularly crucial for spices, such as saffron, paprika, and cinnamon.

1.2.4.1 Saffron

Saffron (*Crocus sativus L.*), one of the most expensive spices globally, is frequently targeted for adulteration and misrepresentation of origin due to its high cost. Several key studies have progressively demonstrated how stable isotope ratios and elemental profiles can verify saffron authenticity, geographical origin, and purity.

The pioneering work by Maggi et al. (2011) provided one of the first comprehensive evaluations of stable isotope analysis for saffron authentication. The authors investigated twenty-eight authentic saffron samples of Crocus sativus L. cultivated in four traditional production regions: Western Macedonia (Greece), Khorasan Province (Iran), Sardinia (Italy), and Castilla-La Mancha (Spain). Each sample was characterized using both chemical quality parameters and stable isotope ratios of hydrogen (δ^{2}H), carbon (δ^{13}C),

and nitrogen ($\delta^{15}N$). Sixteen chemical markers, determined by UV–Vis spectrophotometry, HPLC, and GC analyses, included crocin, picrocrocin, and safranal contents, as well as secondary metabolites such as crocetin esters and 4-ketoisophorone—compounds responsible for saffron's color, bitterness, and aroma. Multivariate analysis based solely on these chemical parameters correctly classified 60.7% of samples by geographical origin.

In contrast, when isotopic data were incorporated, the model achieved 100% correct classification of saffron samples by production region using posterior cross-validation. These results demonstrated that the stable isotope composition of the bio-elements hydrogen, carbon, and nitrogen provides a highly reliable and reproducible fingerprint of geographical origin, reflecting regional environmental and agronomic conditions. The authors noted, however, that additional work across multiple harvest years and other producing countries such as India and Morocco would be necessary to confirm the long-term robustness and transferability of the model.

Building on this isotopic foundation, subsequent research by D'Archivio and co-workers focused on the application of elemental fingerprinting for saffron geographical discrimination. In their 2014 study, the authors analyzed 27 saffron samples from three Italian regions—Abruzzo (L'Aquila), Umbria, and Sardinia—using inductively coupled plasma mass spectrometry (ICP–MS) to assess the potential of mineral composition as a traceability tool. A LDA model based on twelve selected elements (Li, B, Na, Ga, Rb, Sr, Zr, Nb, Cs, Ba, Sm, and Hf) correctly predicted over 80% of samples under leave-one-out cross-validation. Stepwise LDA further identified four key discriminant elements—B, Na, Sr, and Rb—which increased classification accuracy to over 90%. The study demonstrated, for the first time, that mineral composition alone can effectively differentiate saffron originating from distinct Italian production regions, confirming the feasibility of using ICP–MS elemental data for geographic authentication.

A later and more comprehensive study by D'Archivio et al. (2019) extended this approach to compare PDO (Protected Designation of Origin) Zafferano dell'Aquila (Abruzzo, Italy) with Iranian and commercial saffron samples. ICP–MS was again used to quantify thirty elements, and the data were evaluated using both unsupervised PCA and supervised (LDA) multivariate techniques. LDA identified Sr, Ca, Mo, and Fe as the most discriminant elements, enabling clear separation between PDO and non-PDO groups. Class-modeling approaches using UNEQ (Unequal Dispersed Classes) and SIMCA were subsequently applied to validate the PDO category. The UNEQ model achieved 100% sensitivity (all authentic PDO saffron correctly accepted) and 100% specificity (all Iranian and commercial samples correctly rejected), while SIMCA achieved slightly lower but still robust performance (89% sensitivity and 96% specificity). These findings established the elemental signature of Zafferano dell'Aquila as a reliable analytical criterion for confirming product authenticity and geographical origin within the PDO framework.

Together, the 2014 and 2019 D'Archivio studies significantly advanced saffron traceability research by demonstrating that elemental composition can serve as a robust

geographic marker, complementary to stable isotope data. Their work also emphasized that classification models combining multiple discriminant elements (particularly Sr and Rb) can provide rapid, reproducible, and objective tools for origin verification, thus paving the way for integrated isotope–element approaches in subsequent saffron authentication research.

In parallel with these elemental studies, Rocchi et al. (2019) explored the combined use of stable isotope analysis, volatile profiling, and electronic sensing to capture a more comprehensive chemical signature of saffron. A total of 35 samples differing in geographical origin, drying method, and age were analyzed using isotope ratio mass spectrometry (IRMS) for $\delta^{13}C$ and $\delta^{15}N$, solid-phase microextraction gas chromatography–mass spectrometry (SPME–GC–MS) for volatile compounds, and a gold nanoparticle (AuNP)-based electronic nose (E-nose) for aroma fingerprinting. The IRMS results confirmed that $\delta^{13}C$ and $\delta^{15}N$ ratios are reliable discriminants of saffron geographical origin, reflecting underlying climatic and agronomic conditions, while SPME–GC–MS analysis successfully differentiated samples by origin, drying process, and age based on the distributions of aldehydes, terpenes, and carotenoid-derived volatiles. The E-nose, employing a peptide-modified AuNP sensor array, demonstrated partial but promising discrimination of samples by origin, providing a rapid, non-destructive screening tool for routine quality control. The study highlighted the potential of integrating orthogonal analytical approaches—combining isotopic, molecular, and sensorial data—to improve saffron authentication and quality evaluation. However, despite these advances, the authors acknowledged that routine implementation remains limited by the need for larger datasets, standardized reference materials, and inter-laboratory validation to ensure model robustness.

Following these developments, later studies increasingly emphasized the advantages of stable isotope analysis, both at the bulk and compound-specific levels, for identifying saffron origin and detecting adulteration. Seyedghiasi and Parastar (2021) presented a significant advancement by implementing chemometrics-assisted isotope ratio fingerprinting using both an elemental analyzer IRMS (EA–IRMS) and a gas chromatography–combustion IRMS (GC–C–IRMS). A dataset of 62 authentic saffron samples and binary mixtures adulterated with marigold, safflower, rubia, or saffron styles at concentrations ranging from 5% to 35% (w/w) was analyzed for $\delta^{13}C$ and $\delta^{15}N$ isotope ratios. PCA distinguished the two main production regions, while advanced chemometric models—DD–SIMCA, LDA, Quadratic Discriminant Analysis (QDA), and PLS–DA—were used for classification. The QDA model outperformed all others, correctly identifying adulteration at levels as low as 5% with 100% sensitivity and precision, and accurately classifying commercial samples of uncertain authenticity. This work highlighted the superior selectivity and sensitivity of isotope ratio analysis over conventional chemical profiling, particularly for detecting low-level adulteration that cannot be recognized through standard spectroscopic or chromatographic methods.

A related approach was later introduced by Moras et al. (2022), who applied compound-specific isotope analysis (CSIA) using GC–C/P–IRMS to differentiate natural versus

synthetic safranal and to authenticate saffron origin. The $\delta^{2}H$ and $\delta^{13}C$ values of safranal exhibited distinct isotopic signatures for synthetic and natural origins (mean $\delta^{2}H$ of 36‰ vs −210‰, respectively). Furthermore, LDA and QDA models based on $\delta^{13}C$, $\delta^{2}H$, and key saffron metabolites (crocin C2, crocin C3, picrocrocin, and trans-crocetin) achieved 100% calibration accuracy and 82.5% prediction accuracy for distinguishing Iranian, Spanish, and other saffron origins. This study demonstrated how the isotopic composition of specific volatile markers—in this case, safranal—can be used to verify both botanical and geographic authenticity, opening a new dimension in saffron authentication research.

The benefits of combining stable isotope with multi-elemental data were clearly established in the integrated study by Wakefield et al. (2019), who analyzed authentic saffron samples from Iran and Spain using multi-isotope ($\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{34}S$) and elemental (ICP–MS) data. LDA yielded complete discrimination between Spanish and Iranian samples, with isotopic parameters providing confirmatory evidence of climatic origin and elemental data reflecting soil geochemistry. The study emphasized that isotopic tracers, which are directly linked to environmental and physiological processes, offer confirmatory power beyond conventional chemical composition or metabolite-based models.

A major step forward came with the combined isotope–elemental approach of Perini et al. (2020), who analyzed 76 saffron samples from Italy, Iran, Morocco, and commercial markets using ICP–MS (42 elements) together with five stable isotopes ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, $\delta^{2}H$, $\delta^{18}O$). Discriminant analysis identified key isotopic and elemental markers—K, Cr, Mn, Ni, Zn, Rb, Sr, Mo, Cs, Nd, Eu, Pb, $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, and $\delta^{2}H$—that successfully distinguished Italian from foreign saffron. Class models developed for two Italian regions exhibited 100% specificity for Moroccan, Iranian, and commercial samples and >80% specificity for Italian cultivation areas, confirming the effectiveness of integrating isotopic and elemental fingerprints for high-resolution origin verification.

Most recently, Nie et al. (2023) advanced this field by combining elemental (%C and %N) and isotopic data to discriminate between Chinese and Iranian saffron, and among major Chinese production regions. Using Partial Least Squares Discriminant Analysis (PLS–DA), the authors achieved 85% classification accuracy for inter-country discrimination and 80% for regional Chinese classification. The $\delta^{13}C$ and $\delta^{2}H$ values were again identified as the most informative variables, reflecting differences in environmental conditions and cultivation practices. The study demonstrated that harmonized isotope–element approaches can be effectively applied to international saffron markets, providing a basis for standardized regulatory inspection procedures to combat origin mislabeling and strengthen consumer protection.

Taken together, these later studies underscore the growing recognition of isotopic analysis as a superior and confirmatory tool for saffron authentication. While early work focused on elemental or bulk isotope data, newer research demonstrates the enhanced precision achieved through compound-specific isotope ratio analysis and integrated isotope–element datasets. The collective evidence establishes isotopic fingerprinting—whether of

bulk tissue or specific aroma compounds—as a powerful, transferable, and environmentally anchored method for verifying saffron origin, purity, and authenticity. However, challenges remain in developing global isotope–element reference databases, ensuring multi-year calibration stability, and harmonizing analytical protocols across laboratories.

1.2.4.2 Paprika

Among spice commodities, paprika (*Capsicum annuum L.*) has been a central model for testing the combined potential of elemental composition and isotope ratios in origin authentication, particularly because of its economic relevance and the existence of Protected Designation of Origin (PDO) regions in Europe, such as La Vera (Extremadura) and Murcia (Spain), and Szeged (Hungary). As a processed spice, paprika poses unique analytical challenges, since drying, smoking, and grinding can alter its chemical and isotopic composition. Research over the past two decades has therefore focused on identifying robust, process-independent markers of authenticity.

The first major study applying both elemental and isotopic fingerprints was conducted by Brunner et al. (2010), who examined authentic and commercial paprika samples from Hungary and other regions to establish reliable criteria for geographical discrimination. The authors combined strontium isotope ratio ($^{87}Sr/^{86}Sr$) measurements with a comprehensive multi-elemental profile determined by ICP–MS, including trace and rare earth elements (Rb, Sr, Y, Zr, Mo, Cd, Ba, Pb, Th, U, Mg, Ca, Sc, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, As). Using PCA and Canonical Discriminant Analysis (CDA), they achieved clear separation between authentic Szegedi PDO paprika and other commercial varieties. The results revealed that the $^{87}Sr/^{86}Sr$ ratio in paprika reflects the bioavailable strontium fraction of the cultivation soil, which remains conserved through processing. This work provided critical methodological validation proving that isotope–element fingerprints can be used for traceability even in highly processed products such as ground spices.

Building on this isotopic foundation, Palacios-Morillo et al. (2014) extended authentication research to Spanish PDO paprikas from Murcia and La Vera (Extremadura) using a multi-elemental approach via inductively coupled plasma atomic emission spectrometry (ICP–AES). The authors quantified 14 elements (Al, B, Ca, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Sr, and Zn) following acid-wet digestion and validated the method for precision, linearity, and detection limits. They also tested a wide range of pattern-recognition and classification algorithms, including LDA, Linear Support Vector Machines (SVM), Soft Independent Modeling of Class Analogy (SIMCA), and Artificial Neural Networks (ANNs). While linear models effectively distinguished sweet and hot–sweet paprika varieties, the ANN model performed best for geographical differentiation, achieving an overall classification accuracy of 99% independent of paprika type. This finding demonstrated the high predictive capacity of machine learning techniques when applied to multielemental datasets. The authors emphasized that the successful discrimination was linked to the distinct soil and climatic conditions of each PDO region, which are reflected in the mineral composition of the peppers used for paprika production.

In a more recent study, de Jesus et al. (2025) applied a greener, simplified ICP–OES procedure to quantify essential and potentially toxic elements in 25 commercial paprika samples acquired in Brazil. The method employed closed-block digestion using diluted HNO_3/H_2O_2 mixtures and achieved high environmental compatibility with an AGREE greenness score of 0.63. Eleven elements (Ba, Ca, Cu, K, Mg, Mn, Na, P, Sr, V, and Zn) were quantified, and data were analyzed by PCA and Hierarchical Cluster Analysis (HCA). Unlike previous studies, these unsupervised models revealed no clear clustering according to paprika type or declared origin, suggesting uniform mineral composition across samples. The findings reflect the growing challenge of distinguishing paprika origins in globalized supply chains, where commercial blending and standardized industrial processing can obscure natural geochemical signals. Nevertheless, the study underscored the continued relevance of ICP-based elemental profiling as a rapid screening tool for quality monitoring and potential contamination assessment.

Despite significant progress, important gaps remain, particularly in the integration of stable isotopes with elemental data for comprehensive origin verification and the lack of standardized international reference datasets for paprika. Moreover, while statistical classification methods have achieved high discrimination accuracy within national PDO contexts, their transferability across production years and global markets remains limited, reinforcing the need for combined isotopic–elemental approaches like those applied in the present study.

1.2.4.3 Cinnamon

Compared to saffron and paprika, cinnamon (*Cinnamomum spp.*) has been less frequently investigated using stable isotope and elemental analyses for authenticity and origin verification. This limited research attention can be attributed to the complex taxonomy and trade structure of cinnamon, as multiple species (*C. verum, C. cassia, C. burmannii, C. loureiroi*) are marketed under the same commercial name. Additionally, cinnamon products are often sold as ground powders or blends, further complicating efforts to source authentic reference material and verify their true origin (Feltes et al., 2023; di J Pages-Rebull, 2024). Nevertheless, cinnamon remains one of the world's most fraud-prone spices, with adulteration primarily involving the mislabeling of cassia as the higher-value Ceylon cinnamon (*C. verum*) (Ghidotti et al., 2025; Blahová et al., 2012).

The earliest applications of IRMS in cinnamon authentication focused on volatile aroma compounds rather than the whole spice matrix. Fink et al. (2004) applied combustion/pyrolysis isotope ratio mass spectrometry (C/P-IRMS) to determine the δ^2H and $\delta^{13}C$ values of (E)-methyl cinnamate obtained from natural, synthetic, and semisynthetic sources. By comparing isotopic signatures of self-synthesized reference compounds and commercial samples, they demonstrated that synthetic and natural cinnamates could be clearly differentiated. Although no advanced multivariate modeling was applied, the study relied on isotopic range comparison to define authenticity windows,

laying the groundwork for compound-specific isotopic authentication of cinnamon-derived volatiles.

Similarly, Sewenig et al. (2003) analyzed the δ^2H and δ^{13}C isotope ratios of cinnamaldehyde, the key aromatic aldehyde of cinnamon essential oil, using gas chromatography–combustion (GC-C-IRMS) and gas chromatography–pyrolysis (GC-P-IRMS). The authors applied correlation analysis between δ^2H and δ^{13}C values to establish characteristic authenticity clusters for Ceylon, cassia, and wood cinnamon, as well as to differentiate synthetic from natural samples. While multivariate chemometric tools had not yet been integrated, their correlation-based interpretation provided the first isotopic "map" distinguishing cinnamon varieties by their biochemical and geographical origins.

In contrast, Ghidotti et al. (2023) applied a modern multi-elemental profiling approach using energy-dispersive X-ray fluorescence (ED-XRF) combined with multivariate statistical analysis to detect adulteration in commercial cinnamon. Thirty-six elements were quantified across 52 samples (29 Ceylon, 8 cassia, and 15 unspecified), and PCA and hierarchical cluster analysis (HCA) were used to identify grouping patterns and compositional outliers. The models revealed that nearly 60% of samples were adulterated or failed to meet international standards, including products labeled as "Ceylon cinnamon" that were actually cassia or mixed varieties. The findings were further corroborated by headspace GC–MS and thermogravimetric ash analysis, demonstrating the power of combining elemental and spectroscopic data. However, as the study relied entirely on commercial samples with limited traceability and excluded isotopic data, it provided limited insight into the environmental or geographical origin of the material.

Despite these advances, several analytical and methodological gaps persist in the authentication of cinnamon. Earlier research remained focused on isolated volatile compounds or on bulk elemental screening, which do not fully capture the environmental signatures that define botanical or geographical provenance. Chemometric approaches were often basic or absent, and the datasets were typically small and unbalanced, limiting statistical robustness. Moreover, most studies relied on commercial samples of uncertain authenticity, which restricted model validation and reproducibility. Until recently, there had been no comprehensive multi-analytical framework integrating isotopic, elemental, and spectroscopic markers capable of addressing these issues in a unified manner.

1.2.5 IoT and Digital Technologies

Analytical tools and databases alone cannot ensure continuous monitoring; effective traceability also requires real-time tracking and secure information exchange throughout the supply chain. The digital transformation of the food sector has been accelerated through the integration of advanced technologies such as the IoT, AI, blockchain, and other data-driven innovations. The IoT, in particular, refers to interconnected devices—including sensors, RFID tags, and cloud-based systems—that continuously collect and transmit data throughout the supply chain, forming a digital network for real-time monitoring and

intelligent decision-making (Kumar et al., 2019; Figure 5). While the concept of IoT originated in supply chain applications in the late 1990s (Ashton, 2009), it has since evolved into comprehensive, interconnected systems spanning multiple industries.

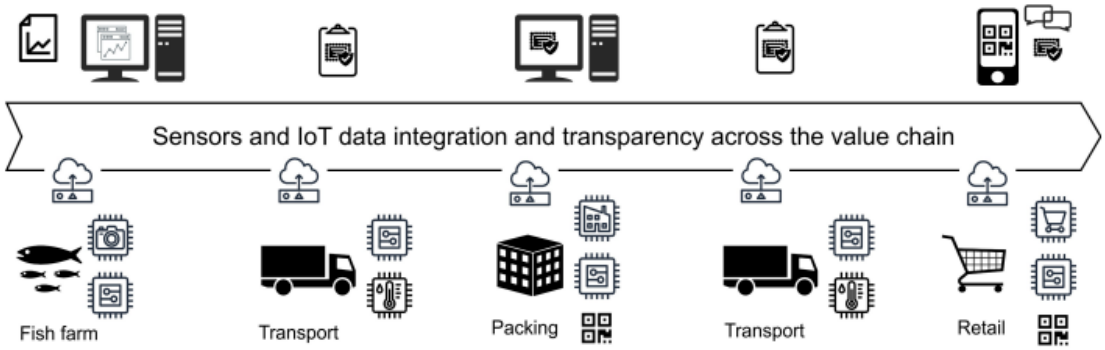


Figure 5: IoT and Sensor Data Integration Across the Fish Supply Chain for Enhanced Transparency (Popović et al., 2024).

Table 3 summarizes the principal advantages and disadvantages of implementing IoT technologies in food systems. Understanding these factors is crucial for stakeholders to effectively leverage IoT for improving supply chain transparency, operational efficiency, and food safety, while also addressing challenges such as data security, system complexity, and cost implications.

Table 3: SWOT analysis of the Internet of Things (IoT) in the food supply chain (Caveen et al., 2021).

Strengths	Weaknesses	Opportunities	Threats
Wide range of applications across the entire food chain	The term "IoT" can be misunderstood or seen as not introducing something new	Increasing use of smartphones as universal interfaces makes IoT technology more accessible	Excessive data generation can be overwhelming
Enables real-time digitization of all aspects of supply chain management and traceability	Many existing devices cannot connect or communicate, requiring replacements	Endless potential applications of sensors, provided investment is made in software and hardware	Overreliance on technology can cause problems during power or internet outages.
Supports faster product recalls by providing information on specific purchased items	Ensuring interoperability and API development between devices can be complex	Enhanced understanding of consumer behavior enables better alignment of food production with market demands	Market competition may push companies to focus on novelty rather than real needs

Provides consumers with greater transparency about their food (e.g., its origin and history)	Dependence on device manufacturers for software and hardware updates	Potential for cost-effective solutions for real-time traceability	Constant need to update and maintain devices
Enhances food safety by identifying issues before they become hazards	Lack of contingency plans if a device fails in a food production environment		Vulnerability to cyber threats (hackers, malware, viruses) requires robust security measures.
Improves inventory management, leading to cost savings	Constant internet access is required, which can be challenging in certain regions or production sites		Data management must comply with privacy and legal requirements
Reduces food waste through better monitoring and control			
Helps prevent or eliminate counterfeit food products			
Improves overall food quality control			

IoT is transforming numerous industries by enabling real-time data collection and intelligent decision-making. In manufacturing, IoT facilitates predictive maintenance through sensors that monitor equipment health, helping anticipate failures, reduce downtime, and lower costs (Lee et al., 2020). Smart agriculture utilizes IoT systems to optimize irrigation and fertilizer use by analyzing real-time soil and climate data, enhancing crop yield and resource efficiency (Obaideen et al., 2022; Alsalami et al., 2025). Meanwhile, cold chain logistics employs smart packaging with integrated temperature and humidity sensors to monitor perishable goods continuously, ensuring safety throughout transport and storage (Pajic et al., 2024; Pal & Kant, 2020).

In food systems, IoT enhances traditional authentication methods by integrating sensors, RFID tags, and wireless communication channels to monitor quality control throughout production, transport, and storage. Continuous monitoring of parameters such as temperature, humidity, and handling conditions helps prevent spoilage and safety failures. At the same time, the associated time–location stamps and product identifiers support the detection of practices such as mislabeling, adulteration, and counterfeiting—critical challenges in ensuring food quality and safety (Bhat et al., 2025; Taj et al., 2023). For example, sensors tracking cold-chain conditions can trigger alerts to prevent spoilage or contamination, transforming traceability from static documentation into a dynamic, evidence-based verification of product integrity. Research confirms that IoT not only optimizes supply chain processes and boosts food safety but also fosters consumer trust

and reduces food waste (Dave, 2025; Cromwell, 2025). For example, smart shelves and bins equipped with sensors can detect when products are nearing their expiry date or when waste levels are increasing, allowing retailers to adjust orders, apply dynamic pricing, or redistribute surplus food before it spoils, thereby cutting avoidable food waste (Ahmadzadeh et al., 2023, Figure 6).

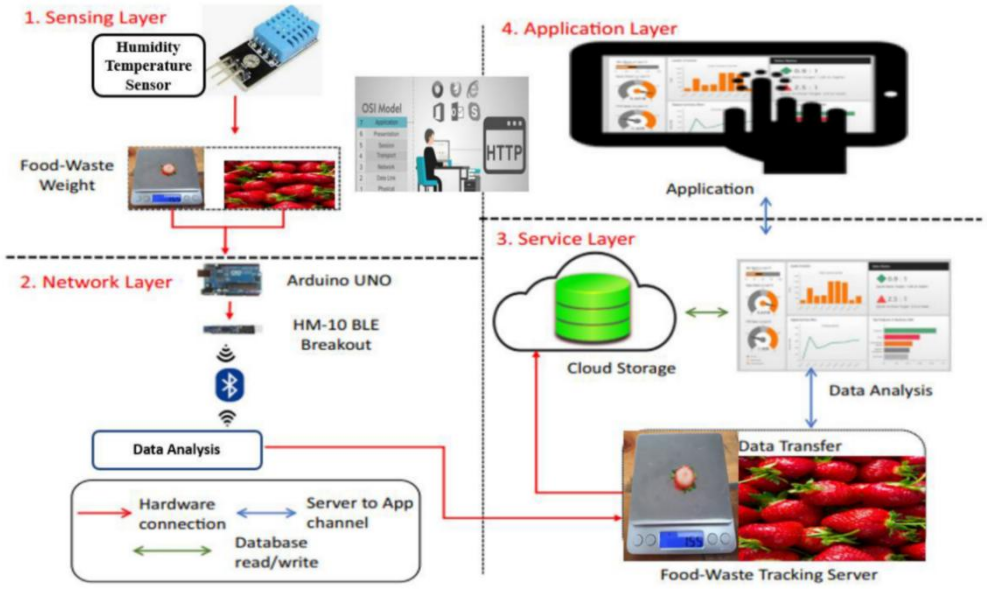


Figure 6: IoT platform for food wastage reduction (Ahmadzadeh et al., 2023).

When combined with blockchain and other digital tools, IoT facilitates secure and tamper-proof data flow across food supply chains through immutable record-keeping (Zhao et al., 2019; Ellahi et al., 2023). This synergy is exemplified in seafood and aquaculture traceability systems, where IoT sensors integrated with blockchain platforms such as Provenance provide comprehensive sea-to-plate visibility for high-value products like tuna and salmon. Provenance collects detailed data at every stage—from catch location and method (via fishermen SMS/apps) to processing and distribution—using blockchain for a secure, transparent ledger (subscription-based, mid-high enterprise cost; combats illegal fishing via QR consumer verification of sustainability/origin) (Provenance, 2024; FAO, 2023).

Similarly, SourceTrace's TraceNext employs IoT sensors for real-time spice farm monitoring (temperature/humidity) alongside blockchain, capturing cultivation-to-market data to overcome quality barriers and enable premium certifications (paid SaaS, costly for small farmers but offsets via market access) (Caveen et al., 2021; SourceTrace, 2020). A related development in aquaculture is the use of cloud-based SaaS platforms that integrate IoT sensors for real-time monitoring and data exchange across fish farms, transport, and retail chains, improving interoperability and supporting the detection of integrity issues such as mislabeling and fraud (Popović et al., 2024).

Comparable developments can be found across other food sectors. For instance, Beefchain™ applies blockchain-based smart tagging to trace the origin and production of

grass-fed beef in Wyoming, enabling ranchers to obtain price premiums while offering consumers verifiable assurance of authenticity and animal welfare (Caveen et al., 2021). In the baking sector, the Digital Sandwich project in the United Kingdom integrates IoT sensors, blockchain, and artificial intelligence to track ingredient flows within multi-component food products, thereby strengthening allergen management and improving oversight across processing steps (Caveen et al., 2021). Likewise, the IBM Food Trust initiative connects major brands such as Nestlé, Unilever, and Walmart through a shared blockchain network that supports rapid verification of product batches and improves supply-chain visibility and safety (Caveen et al., 2021).

Chapter 2

Aims and Hypotheses

The complexity of the global food supply chain and the increasing economic incentives for fraud present significant challenges to ensuring product authenticity and traceability. Fraudulent practices undermine consumer trust, distort market dynamics, and compromise sustainable business practices. Addressing these challenges effectively requires a multidisciplinary response, as no single technology or method alone is sufficient to guarantee authenticity across biological, chemical, digital, and logistical dimensions simultaneously. Analytical methods such as stable isotope and multi-elemental analysis, combined with the development of structured databases and advanced chemometric approaches, have emerged as powerful tools for verifying the origin and integrity of food products. However, despite these advancements, the lack of standardized reference databases and the growing sophistication of fraudulent practices continue to limit the effectiveness of existing verification methods. The four objectives of this thesis were therefore defined and sequenced according to a deliberate methodological logic: progressing from analytically established approaches toward emerging digital technologies, and from foundational data infrastructure toward advanced predictive modeling, so that each objective builds upon and reinforces the preceding one.

The first objective of this thesis was to conduct stable-isotope and multi-elemental analyses of saffron, paprika, and cinnamon to determine their geographical origin and production type (conventional versus organic). These spice commodities, chosen as case studies, are highly susceptible to fraud and mislabeling due to their high economic value and protected designation of origin status, and their characterization will contribute to a more reliable authentication framework. This objective was prioritized first because stable isotope ratio analysis and elemental profiling are analytically well-validated, high-precision methods that provide the robust reference data upon which the subsequent objectives depend.

To address the lack of a comprehensive standardized database, the second objective was to further develop and expand IsoFoodTrack, a web-based authentication platform, by incorporating additional isotopic and elemental data along with relevant metadata such as geographic origin, production conditions, and seasonal variations. This expanded database will serve as a standardized reference framework for food authenticity research and support continuous scientific collaboration and data sharing. This objective was pursued concurrently with the first, as authentication is only as reliable as its underlying

reference framework — high-quality analytical data generated without a structured, interoperable database would be of limited long-term scientific utility.

Besides building a robust data repository, there is also a pressing need to improve statistical evaluation tools for fraud detection. Traditional approaches, such as discriminant analysis, have limitations for classification in authenticity studies, particularly when datasets are imbalanced, sample sizes are small, or when interpretability of classification decisions is required for regulatory purposes. Therefore, the third objective was to apply advanced statistical modeling, including OPLS-DA and one-class classification methods like DD-SIMCA, and to enhance these methods with artificial intelligence and machine learning algorithms. These tools will allow for more accurate and scalable predictions regarding food authenticity and fraudulent patterns. This objective was placed after the first two because both advanced statistical models and machine learning algorithms require well-curated, representative datasets to generalize reliably; the IsoFoodTrack database developed in the preceding objectives provided the necessary data foundation for robust model training and validation.

While physical verification methods provide strong evidence for authenticity, digital traceability technologies offer complementary capabilities by monitoring supply chains in real time. For this reason, the fourth objective was to integrate the Internet of Things (IoT) for seafood traceability by deploying a fish farm management platform in collaboration with DigitalSmart. The mini-pilot study, conducted in Montenegro, involves real-time monitoring of aquaculture parameters (e.g., water quality, feeding) and cold-chain tracking during transport (GPS location, temperature, and humidity). This objective was intentionally scoped as a proof-of-concept pilot, as IoT-based digital traceability in food systems remains an emerging field; the aim was to demonstrate feasibility and establish the integration pathway between physical verification and dynamic, real-time supply chain monitoring.

Together, these four objectives form a coherent, layered framework that moves from physical evidence generation, through data infrastructure, to intelligent modeling, and finally to real-time digital transparency, reflecting the multidisciplinary nature of the challenge they address.

This thesis tests the following hypotheses:

- H1: Stable isotope and elemental analysis can successfully differentiate between conventional and organic production, as well as verify the geographical origin of saffron, paprika, and cinnamon.

- H2: The developed method could be used to assess the authenticity of commercial spices on the market, with the expectation that more than 80% of spices are correctly labelled.

- H3: The integration of AI-driven verification methods improves the classification accuracy of food authenticity compared to traditional statistical modeling.

- H4: IoT-enabled real-time monitoring of fish farms (DO, ORP, pH, EC, water temperature) enhances aquaculture management and ensures seafood quality.

2.1 Structure of the Scientific Part of the Thesis

To assess the proposed hypotheses, the scientific part of this doctoral thesis is divided into three interconnected sections (Figure 7).

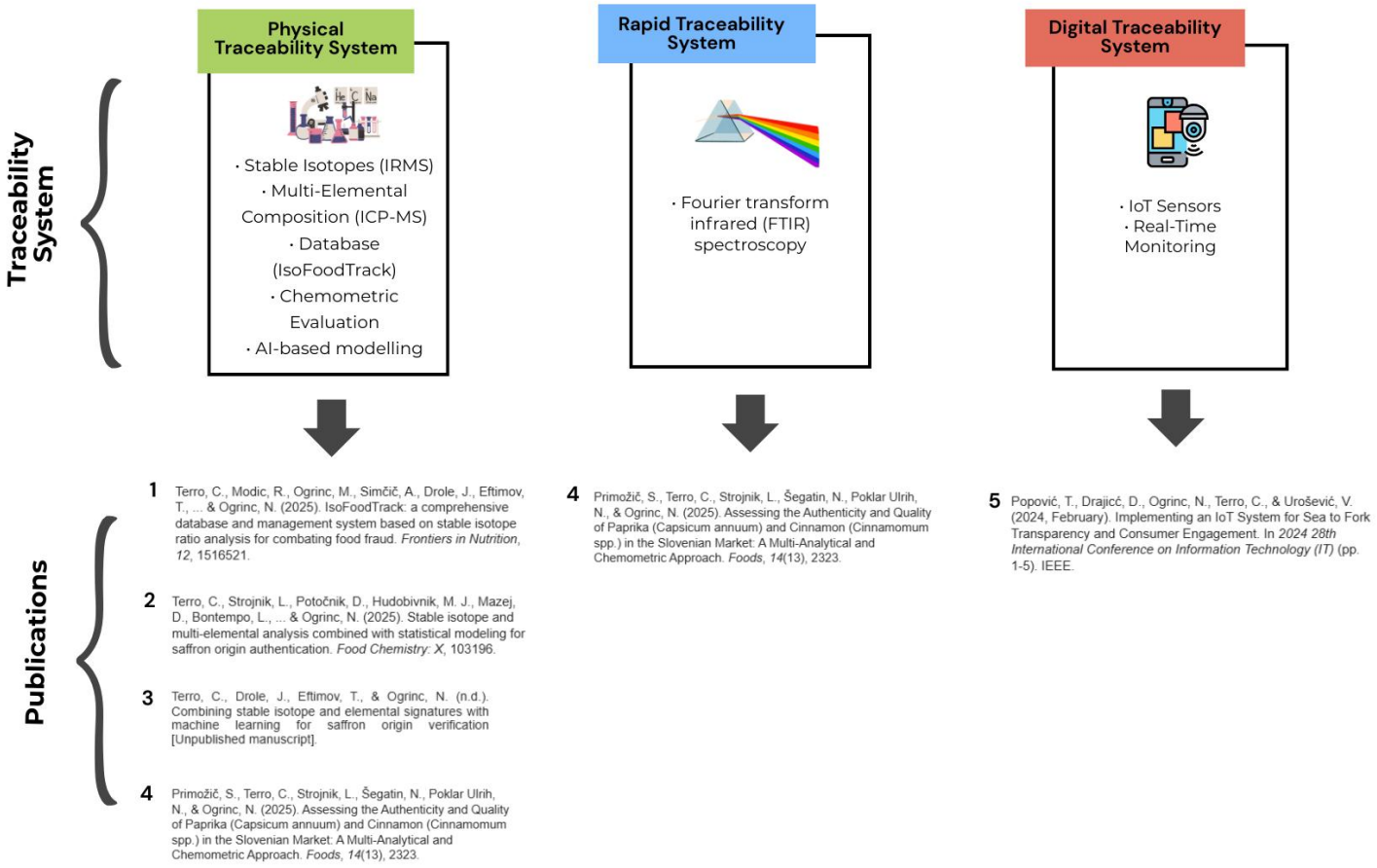


Figure 7: Scientific structure of the thesis.

Figure 7 illustrates the narrative arc of my research and the rationale behind the sequence of publications, showing how physical, rapid, and digital traceability systems were progressively integrated across multiple food case studies, including saffron, paprika, cinnamon, and seafood. The doctoral research began with a clear goal: to strengthen food authenticity and traceability by developing innovative, scientifically robust tools to ensure the integrity of the food supply chain. This led to the adoption of a physical traceability system, which forms the backbone of the thesis and is documented in **Publications 1–4**.

The first step addressed the lack of a comprehensive, standardized food authenticity database. **Publication 1** introduced the IsoFoodTrack database, designed to store stable isotope and multi-elemental composition data along with rich metadata, including geographical, production, and methodological details. Recognizing the need to populate the

database with high-value, fraud-prone commodities, saffron was selected. Isotopic and elemental data from authentic saffron samples were integrated into IsoFoodTrack, along with the metadata, providing a foundation of high-quality, traceable information that inspired the next phase of research.

Building on this, **Publication 2** focused on developing a robust approach for saffron origin authentication. Stable isotope and multi-elemental analyses (via IRMS and ICP-MS) were combined with statistical modelling (OPLS-DA, DD-SIMCA) to distinguish saffron samples from multiple countries and detect mislabeling. **Publication 3** extended this work by integrating AI-driven verification methods, evaluating whether machine learning improves the classification accuracy of food authenticity compared to traditional statistical modeling.

Recognizing the need for faster and more cost-effective screening, the research then expanded into rapid traceability. **Publication 4** introduced FTIR spectroscopy as a rapid analytical tool for authenticity assessment in paprika and cinnamon, providing a faster alternative in addition to IRMS and ICP-MS, complementing the more detailed but time-intensive physical analyses.

Finally, to address traceability beyond laboratory analysis, the thesis incorporated a digital traceability system. **Publication 5** demonstrated how IoT-based monitoring and digital integration can enable real-time, sea-to-fork transparency in seafood supply chains, linking producers and consumers through an end-to-end digital platform.

Together, these **five** publications reflect a deliberate progression from robust physical authentication to rapid screening and ultimately to digital transparency, forming a coherent and holistic traceability framework.

Chapter 3

Publications

3.1 Scientific Paper: "Isofoodtrack: A Comprehensive Database and Management System Based on Stable Isotope Ratio Analysis for Combating Food Fraud"

In this chapter, I present the paper entitled "IsoFoodTrack: a comprehensive database and management system based on stable isotope ratio analysis for combating food fraud" by Cathrine Terro, Robert Modic, Matevž Ogrinc, Andraž Simčič, Jan Drole, Tome Eftimov, Barbara Koroušić Seljak, and Nives Ogrinc. The paper was published in Frontiers in Nutrition (Nutrition Methodology) in 2024 as part of the Databases and Nutrition, Volume III research topic.

IsoFoodTrack manages isotopic and elemental composition data for a wide range of food commodities, integrating stable isotope ratio data with rich metadata, including geographical, production, and methodological details. Built for scalability, the database supports continuous addition of new commodities, analytical methods, and metadata fields while ensuring interoperability with external databases through standardized formats and API integration.

The platform uses statistical, chemometric, and machine learning approaches to classify the origin of food commodities and detect fraud. IsoFoodTrack also enables isotope mapping (isoscapes), providing spatial predictions that improve detection of adulteration. Its rigorous quality control measures ensure high data reliability, and the user-friendly web interface facilitates easy access and visualization. IsoFoodTrack is openly accessible through platforms such as the National Center for Biomedical Ontology (NCBO) BioPortal and is positioned for future expansion and integration with open-access data, making it a vital tool for researchers and regulatory agencies.

In this study, I was responsible for collecting data from both experimental laboratory samples and conducting a literature review. I also organized and curated the data for inclusion in the IsoFoodTrack database. Additionally, I acquired and systematically structured relevant metadata, which complemented the primary data and enhanced the overall dataset for further analysis. My role also included manuscript preparation and writing.

The work was presented at six international conferences both orally and as a poster, including the International Symposium on Food Safety and Control (May 2024, Vienna, Austria), the International Mass Spectrometry Conference (IMSC) (August 2022, Maastricht, The Netherlands, and August 2024, Melbourne, Australia), the ISO-FOOD Symposium (April 2024, Portorož, Slovenia), and the Jožef Stefan International Postgraduate School Students' Conference (June 2022 and May 2023, Kamnik, Slovenia). I received the **Best Contribution Award** (poster and oral presentations related to this work) at the 15th Jožef Stefan IPSS Conference (2023). Additionally, this work was presented during the FoodTraNet Project meeting and Winter School (March 2024), held at Aristotle University of Thessaloniki (AUTh) in Thessaloniki, Greece, as well as during the FoodTraNet Project meeting and Summer School (June 2023), held at the Experimental Station of Arid Zones (EEZA) of the Spanish National Research Council (CSIC) in Almería, Spain.

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IsoFoodTrack: a comprehensive database and management system based on stable isotope ratio analysis for combating food fraud

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The IsoFoodTrack database is a comprehensive, scalable, and flexible platform designed to manage isotopic and elemental composition data for a wide range of food commodities. It supports research in food authenticity and fraud detection by integrating isotopic data with rich metadata, including geographical, production, and methodological details. The database is built for scalability, allowing the addition of new commodities, analytical methods, and metadata fields, while ensuring interoperability with external databases through standardized formats and API integration. Based on the data collected in IsoFoodTrack using statistical, chemometric and machine learning approaches it has a capability to identify and classify the origin of food commodities. IsoFoodTrack also supports isotope mapping (isoscapes), providing spatially continuous predictions that enhance the detection of food fraud. Rigorous quality control measures ensure high data reliability, and the user-friendly web interface facilitates easy access and visualization. Openly accessible through platforms like National Center for Biomedical Ontology (NCBO) BioPortal, IsoFoodTrack is positioned for future expansion and integration of open-access data, making it a vital tool for researchers and regulatory agencies in ensuring food authenticity and traceability.

KEYWORDS

database, stable isotope ratio analysis, elemental composition, food fraud, authenticity, interoperability

1 Introduction

Food fraud, which refers to the economically motivated adulteration and mislabeling of food products, continues to be a major issue for food producers as well as consumers. Among the techniques available for detecting fraud, stable isotope fingerprinting is leading the way in establishing the authenticity and geographical origin of food products. This choice is based on the fact that the distribution of stable isotopes of carbon (^{12}C, ^{13}C), nitrogen (^{14}N, ^{15}N), sulfur (^{32}S,

^{34}S), hydrogen (^{1}H, ^{2}H), and oxygen (^{16}O, ^{18}O)[1] is influenced by fractionation processes linked to local climate, geology, and soil characteristics (1, 2). These processes result in varying rates of isotope transfer from natural sources such as water, soil, and the atmosphere to plant or animal tissues. For example, the isotope ratios in water (^{2}H/^{1}H and ^{18}O/^{16}O) provides critical information about local precipitation, surface water, and groundwater, influenced by factors like latitude, altitude, distance from the sea, precipitation levels, and evapotranspiration. The verification of regional origin becomes even more robust when isotope data are combined with elemental composition profiles (3). However, to determine authenticity, a suitable reference dataset of analyzed authentic products is required. This dataset should include samples representative of a wide range of geographical, seasonal, dietary, and production conditions. Authenticity is then assessed by comparing the values found in commercial samples with the limits estimated from the reference dataset, using a suitable statistical model to evaluate the best fit. These databases also need to be continuously curated and kept up to date, which is a considerable task, given the amount of variation that needs to be included.

A prime example of a well-established database is the European Wine DataBank, which the European Commission has maintained for over 20 years (4). However, while some databases are publicly available, many others are not freely shared due to intellectual property concerns and differences in sample pre-treatment methods. Nevertheless, the use of isotope databases is expanding; for example, the Stable Isotope Ratio Analysis (SIRA) database is already being applied to products like Parma ham, Grana Padano cheese, and Parmigiano Reggiano in Italy (5). Other examples include the pork origin database managed by the UK's Agriculture and Horticulture Development Board (AHDB), the egg database Kontrollierte Alternative Tierhaltungsformen (KAT), and asparagus databases in German food control labs.

To address the gaps in the availability and accessibility of such data, we have developed a comprehensive database and database management system (DBMS) called IsoFoodTrack.[2] This system provides extensive data on the stable isotopes of light elements and the elemental composition of authentic samples from various food commodities such as oils, milk and dairy products, meat, spices, truffles, seafood and vegetables. Furthermore, IsoFoodTrack is designed to be interoperable, allowing connection with other databases or centralized repositories. IsoFoodTrack represents a significant advancement over traditional food databases by prioritizing both accessibility and standardization. It incorporates open-access principles, ensuring that researchers from diverse regions can utilize its resources without significant barriers. Additionally, the database integrates standardized metadata protocols and harmonized data entry formats, which streamline cross-study comparisons and enhance reproducibility.

Additionally, there is a growing movement toward creating a centralized repository for isotopic data, as proposed by Pauli et al. (6), with the development of IsoBank. IsoBank aims to function similarly to GenBank in the field of genetics, serving as both an aggregator and repository of open-access isotope data. IsoBank is designed as a general-purpose repository for stable isotope data across all disciplines. It supports the storage and retrieval of isotope measurements irrespective of their context. It serves a broad research community, including fields like ecology, geology, archeology, and biology, among others. This resource will promote interdisciplinary research, facilitate data-sharing, and provide valuable educational opportunities by offering real-world isotopic data for students and researchers alike. On the other hand, IsoFoodTrack database serves as a specialized database aimed at practical applications in food traceability and authenticity verification, prioritizing functionality tailored to its specific use case. Its scope is narrower, targeting applications in food science, agriculture, and regulatory frameworks.

In this paper, we present the IsoFoodTrack database as the first effort to organize open-access stable isotope data for food research. It is organized in different sections including: database design, methods and technical aspects. Section 5 details the validation of IsoFoodTrack, demonstrating its practical application, while section 6 deals with database curation and availability. Finally, section 7 concludes the paper by discussing key achievements and contributions.

2 Database design

The design of the IsoFoodTrack database is crucial for ensuring the effective management of isotopic and elemental composition data for various food commodities. The database was developed with a focus on scalability, flexibility, and data integrity, enabling researchers to store, retrieve, and analyze stable isotope data in a structured and efficient manner. This section outlines the key aspects of the database design, including the data model, schema design, relationships between entities, and considerations for maintaining accuracy and performance.

2.1 Data model

The IsoFoodTrack database follows a relational database model, which is well-suited for managing structured data with clearly defined relationships between entities. The relational model enables efficient querying of data, as well as maintaining consistency and data integrity through the use of primary and foreign key constraints. This design ensures that all data points, including isotopic ratios, elemental compositions, and metadata about samples, are properly linked to their relevant entities.

The structure of IsoFoodTrack is presented in Figure 1.

The main entities in the database include:

- *Samples*: representing the physical food samples analyzed for isotope and elemental composition. This also includes the type of

1 Measurements of the stable isotope ratios of light elements are expressed in the δ-notation in ‰ according to the equation: δE = $(R('E/E)_{sample}/R('E/E)_{standard}) - 1$, where *i* stands for the highest and *j* for the lowest atomic mass number of the element E (C, N, O, S), and R is the isotope ratio between the heavier and the lighter isotope of the element (^{2}H/^{1}H, ^{13}C/^{12}C, ^{15}N/^{14}N, ^{18}O/^{16}O, ^{34}S/^{32}S) in the *sample* or *standard*. The δ^{13}C values are expressed relative to V-PDB (Vienna-Pee Dee Belemnite) standard, δ^{15}N values relative to AIR, δ^{34}S values relative to V-CDT (Vienna-Canyon Diablo Troilite) standard and the δ^{2}H and δ^{18}O values relative to the VSMOW (Vienna-Standard Mean Ocean Water) standard.

2 http://isofoodtrack.ijs.si

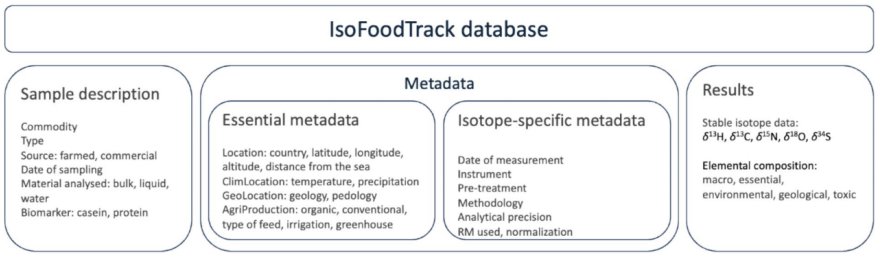


FIGURE 1
Structure of the IsoFoodTrack database.

sample, source of sample (authentic, commercial), date of sampling and compound analysed: bulk sample (freeze-dry or liquid), sample water, extracted components, fatty acid.

- *Metadata*: the metadata cover two categories: (i) essential metadata, describing every data record, and (ii) isotope-specific metadata. The essential metadata includes *geographical data:* storing information about the geographical location of each sample, including details such as latitude, longitude, altitude, distance from the sea. In addition, the data on yearly average amount of precipitation, average temperature of the location, geology and pedology are also included.

Production data: capturing details about the production and processing of the food samples, such as year of production, type of material (authentic, commercial), farming practices, seasonal information, and production methods (e.g., organic or conventional; if known).

The *isotope-specific metadata* includes reference materials used to normalize the data. Stable isotope data are produced in a wide range of research and commercial laboratories. Although the methods by which the majority of data, i.e., bulk carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope values, are standardized, laboratories often use slightly different protocols and different laboratory reference materials to normalize data to internationally accepted scales. Other isotopes (e.g., $\delta^{2}H$ and $\delta^{18}O$) have more fundamental issues associated with comparability of measurements (7). Hydrogen in an exchangeable position (e.g., when bound to oxygen in a hydroxyl group, as in proteinaceous material) will exchange with atmospheric water vapor, leading to potentially erroneous results unless controlled. Thus, to ensure data robustness, quality and user confidence, all pertinent analytical information for each piece of data is recorded. IsoFoodTrack metadata includes sample pretreatment methods (e.g., lipid extraction), reference materials used, type of normalization (one, two, multi-point normalization) and instrumentation used.

- *Isotope data*: storing detailed information about the isotopic composition of each sample, including ratios of isotopes such as $\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{34}S$ (in ‰).
- *Elemental data*: capturing elemental concentrations for key elements: B, Na, Mg, Al, P, S, K, Ca, V, Cr, Mn, Fe, Co, Cu, Zn, As, Se, Rb, Sr., Mo, Cd, Cs, Ba, Hg, Pb.

2.2 Scalability and interoperability

The design of IsoFoodTrack anticipates the continuous expansion of the database as new samples are collected and analyzed. As such, the database architecture is scalable, with the ability to accommodate additional tables for new food commodities or analytical methods such as compound specific analysis.

3 Methods

The development of the IsoFoodTrack database involved several key stages, including the collection and preparation of authentic food samples, the analysis of isotopic and elemental compositions, the organization and management of data, and the validation of the database for practical applications in food authenticity testing. This section describes the methodology employed to create and curate the IsoFoodTrack database.

3.1 Sample collection and preparation

Sample collection represents a crucial step in the formation of the IsoFoodTrack database, and in order to ensure the accuracy of a food authenticity database, authentic samples must be used. Ideally, samples should be collected from primary producers by impartial collectors to ensure traceability and authenticity. In comparison, retail samples are less reliable due to possible contamination in the supply chain. When creating the database, sample size and variety are also important and should reflect natural variations, e.g., geography, breed, and climate. Additionally, the database should be validated for its intended use, and statistical analysis should be considered when determining sample size. The selection of reference data from the database is crucial and should be left to experts only (8, 9).

In our case, the sampling follows an appropriate protocol developed for various applications to mitigate potential biases caused by the overrepresentation of specific regions. This protocol is based on our prior experience and expert knowledge. To ensure a robust and representative dataset, samples were collected using the following criteria:

Geographical diversity: samples were sourced from a wide range of geographical locations, including different latitudes, altitudes, and climatic zones. This ensures that the database captures the natural variation in isotopic and elemental signatures that arise from environmental factors such as local precipitation, soil types, and temperature. In case of Slovenia, to account for natural variability, sampling was designed to reflect Slovenia's regionalization, which is divided into four distinct geographical regions: Dinaric, Mediterranean, Alpine, and Pannonian.

Seasonal and temporal variation: samples were collected over multiple growing seasons and harvest periods to account for seasonal changes in isotopic and elemental composition. These variations can be influenced by factors such as precipitation levels and temperature fluctuations throughout the year.

Production methods: both conventional and organic food production methods were represented in the sample set.

It is also useful to understand the production density of a foodstuff and the number of relevant authentic samples. For example, the Slovenian wine database, established in 1996, contains 25 authentic wine samples covering various geographical regions and varieties. The database is also included in EU Wine Databank. Another example is related to verification of correct labeling of selected fruits and vegetables on Slovenian market including strawberries, cherries, asparagous apples, kaki and garlic. Authentic samples are provided annually by the regional units of the Administration of the Republic of Slovenia for Food Safety, Veterinary, and Plant Protection from producers from various geographical production areas. This research began in 2018 and requires at least 30 authentic samples covering four geographical regions.

Once collected, each sample was carefully labeled with metadata, including information on the geographic origin, date of collection, food type, and any relevant production details. Samples were then prepared for isotopic and elemental analysis according to standardized operational protocols, ensuring consistency in the treatment of all samples.

Although IsoFoodTrack has been designed to cover only a limited area, such as Slovenia, it is crucial to recognize the limitations inherent in regions with sparse data availability on a global scale. Low-data regions may exhibit incomplete coverage, which can limit the robustness of isotopic analysis in those areas. To address this, IsoFoodTrack could adopt the following two strategies to enhance its global applicability: encouraging contributions from other researchers, initiatives that can fill data gaps and build regional datasets and leveraging artificial intelligence (machine learning) to predict isotopic baselines in low-data regions, taking into account appropriate uncertainty metrics.

3.2 Isotopic and elemental analysis

The analysis of stable isotope ratios and elemental composition was conducted using precise and well-established techniques. The two main analytical methods used were Isotope Ratio Mass Spectrometry (IRMS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) for elemental profiling.

3.2.1 Isotopic analysis

There are only a few exceptions where no sample treatment is needed, such as determining the $\delta^{18}O$ value of water in food and the $\delta^{2}H$, $\delta^{13}C$, and $\delta^{18}O$ values of olive oil. However, in most cases, sample treatment is required since it permits the isolation of components that have a stronger geographical fingerprint than the bulk sample and with less interference. For example, samples containing lipids (e.g., meat, fish, milk, and cheese) are usually defatted because fat has a different C and H composition from the other food constituents, and therefore, its variable quantity can affect the overall isotopic signature. In the IsoFoodTrack samples, defatting was performed using a mixture of petroleum ether and diethyl ether (2:1 v/v). Before analysis, all fractions were freeze-dried and stored at room temperature.

Isotope ratios of hydrogen ($^{1}H/^{2}H$), carbon ($^{12}C/^{13}C$), nitrogen ($^{14}N/^{15}N$), oxygen ($^{16}O/^{18}O$), and sulfur ($^{32}S/^{34}S$) were measured using an isotope ratio mass spectrometer (IRMS) with different preparation systems. The solid and liquid samples are measured by elemental analyser coupled to IRMS (EA/IRMS), $^{1}H/^{2}H$ and $^{16}O/^{18}O$ in organic matrices with a TC/EA pyrolyser coupled to IRMS, while $^{1}H/^{2}H$ and $^{16}O/^{18}O$ in water in food samples is determined with MultiFlow Bio equilibration system connected to IRMS.

Validation of a database includes the data within it and its ability to complete the role for which it was designed. All data used to create the database must be validated, i.e., reliable, and all measurements must follow the protocol suggested by Skrzypek et al. (10). The validation tests highlighted in the manuscript were instrumental in assessing its reliability. These tests revealed occasional false positives and misclassifications during bivariate evaluations, particularly in food samples with isotopic compositions near boundary thresholds. For example, foods sourced from regions with similar climatic and environmental conditions exhibited overlapping isotopic signatures. To address these issues, enhanced multivariate analyses were implemented to improve classification accuracy, reducing false-positive rates to below 5% in most categories.

Further, if, upon re-analysis of the samples, data that are consistent with the initial "outlier" data are recorded, further investigation are undertaken to determine the underlying cause. Typically, outliers are due to particular and unique technological or geographical issues, such as a particular microclimate or technological choice. In this case, further investigations are carried out to understand if the outliers belong to another population of data or if they are just "outliers" falling in the percentage of error of the chosen confidence level (for example, 5% for 95% confidence level).

It is strongly recommended that laboratories are accredited to ISO17025 or can demonstrate that they have equivalent quality control systems. This is specifically required if we use the databank to verify the authenticity of commercial samples for food control purposes. According to the norm EN ISO/IEC 17025, the test result of an analytical measurement has to be stated with an estimate of its uncertainty, for example, when uncertainty affects compliance with an authenticity limit. Measurement uncertainty is usually reported in the reference methods (in the case of official and validated methods), or it can be estimated using different methods. Dunn et al. (11) recently published guidance for calculating measurement uncertainty of stable isotope ratio delta values. A further requirement of accreditation is that laboratory must participate in proficiency testing that comply with the ISO/IUPAC/AOAC International Harmonized Protocol for Proficiency

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Testing of analytical laboratories. In our case this involves participation in the Food Analysis using Isotopic Techniques Proficiency Testing Scheme (FIT PTS), organized by Eurofins Scientific three times per year and includes samples from various food commodities.

3.2.2 Elemental analysis

The elemental composition of each sample was analyzed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS), which is highly sensitive to trace elements. The elemental composition in our samples was performed on triple quadrupole inductively coupled plasma mass spectrometer, QQQ-ICP-MS (Agilent, USA). Prior to analysis, the samples were digested using appropriate chemical methods (e.g., acid digestion) to ensure the accurate quantification of elemental concentrations. Detection limits included in the table were calculated based on three standard deviations of blank measurements. A more detailed description of the optimization of the method for elemental analysis for fruits and vegetables can be found in (12). The elemental data were recorded as concentrations (mg/kg or ppm) for key elements that are relevant to distinguishing different geographical origins and production methods.

Both isotopic and elemental data are stored in the IsoFoodTrack database, alongside the associated sample metadata, to allow for comprehensive comparative analyses. In IsoFoodTrack, isotopic and elemental composition data are represented as single-point measurements.

4 Technical aspects

The construction of the database involves three main phases:

- Phase I: the database structure definition. The protocol for data preparation was evolved to minimize the labor involved in populating the database. The database also includes metadata that are important for the further evaluation of the data.
- Phase II: filling the database with data gained during the project evolution.
- Phase III: development of routines/queries for extracting data from the database.

The fundamental requirements of IsoFoodTrack included (i) the underlying database and (ii) the application layer (web application).

4.1 Underlying database

The following points were considered in the underlying database:

Database platform: the IsoFoodTrack database was implemented using PostgreSQL, an open-source relational database system known for its robustness, support for complex queries, and ability to handle large datasets. The specific technology was chosen for its reliability and synergy with other web technologies. PostgreSQL also enables the storage of semi-structured data when necessary.

Data import and export: bulk data import and export were handled using python scripts with PostgreSQL connectors. Data entry was streamlined by importing the isotopic and elemental data from excel file.

Underlying database schema is flexible to mitigate the need for further modification to accommodate growing metadata or analytical results requirements.

A detailed visualization of the database's structure is provided in the Supplementary Figure 1. This visualization includes an Entity-Relationship (ER) diagram that maps out the database's tables, the relationships between these tables, and the attributes that define each entity. The diagram identifies primary keys, foreign keys, and various constraints, providing a thorough overview of how data points are linked, referenced, and maintained across the entire IsoFoodTrack ecosystem.

4.2 Web application

The web application is a user-friendly interface for interacting and accessing the data from the IsoFoodTrack database. The IsoFoodTrack user interface was built using Django (a Python-based web framework), which allows for integration with the PostgreSQL database. The landing page (Figure 2) is designed to allow users to access the data quickly. All information is organized into categories, and when selecting a category (isotope, elemental composition), a tabular display is presented with the relevant data (Figure 3). The data of elements are grouped into macro, essential, environmental, geological, and toxic categories.

The following aspects were considered for the application:

- Security and Auditing (created, updated): The database is accessible to the public only via the web application. Once all data is published, access will be extended to interested users.
- Configuration or metadata and results: Key information, such as column names, data type, units, location data and categorization, are included in the database (Figure 3).
- Review process: Before any new results are added to the database, they are reviewed by the administrator and experts.
- Data Visualization: Django's ORM (Object-Relational Mapping) was used to query the database and display the results in a user-friendly format. Visualization of data was done using an interactive map with data points representing isotope measurements on different food items from various places around the world (Figure 4). Each data point is clickable and contains relevant information for easier comparison.

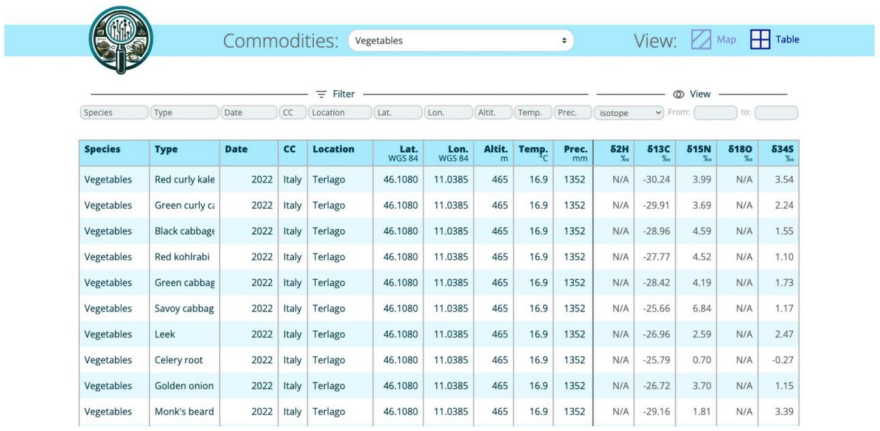
4.3 Interoperability

Leveraging the data interoperability within the IsoFoodTrack database, an API (Application Programming Interface) can be implemented to allow other systems to interact seamlessly with IsoFoodTrack. This permits external systems or researchers to query the database and retrieve data in standardized formats such as JSON or CSV. The use of standardized data formats and metadata conventions ensures compatibility, facilitating data exchange and cross-referencing with other isotope databases or centralized repositories, like IsoBank.

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FIGURE 2
Interface of IsoFoodTrack database.



Species	Type	Date	CC	Location	Lat. WGS 84	Lon. WGS 84	Altit. m	Temp. °C	Prec. mm	δ2H ‰	δ13C ‰	δ15N ‰	δ18O ‰	δ34S ‰
Vegetables	Red curly kale	2022	Italy	Terlago	46.1080	11.0385	465	16.9	1352	N/A	-30.24	3.99	N/A	3.54
Vegetables	Green curly c	2022	Italy	Terlago	46.1080	11.0385	465	16.9	1352	N/A	-29.91	3.69	N/A	2.24
Vegetables	Black cabbage	2022	Italy	Terlago	46.1080	11.0385	465	16.9	1352	N/A	-28.96	4.59	N/A	1.55
Vegetables	Red kohlrabi	2022	Italy	Terlago	46.1080	11.0385	465	16.9	1352	N/A	-27.77	4.52	N/A	1.10
Vegetables	Green cabbag	2022	Italy	Terlago	46.1080	11.0385	465	16.9	1352	N/A	-28.42	4.19	N/A	1.73
Vegetables	Savoy cabbag	2022	Italy	Terlago	46.1080	11.0385	465	16.9	1352	N/A	-25.66	6.84	N/A	1.17
Vegetables	Leek	2022	Italy	Terlago	46.1080	11.0385	465	16.9	1352	N/A	-26.96	2.59	N/A	2.47
Vegetables	Celery root	2022	Italy	Terlago	46.1080	11.0385	465	16.9	1352	N/A	-25.79	0.70	N/A	-0.27
Vegetables	Golden onion	2022	Italy	Terlago	46.1080	11.0385	465	16.9	1352	N/A	-26.72	3.70	N/A	1.15
Vegetables	Monk's beard	2022	Italy	Terlago	46.1080	11.0385	465	16.9	1352	N/A	-29.16	1.81	N/A	3.39

FIGURE 3
The tabular display of a category.

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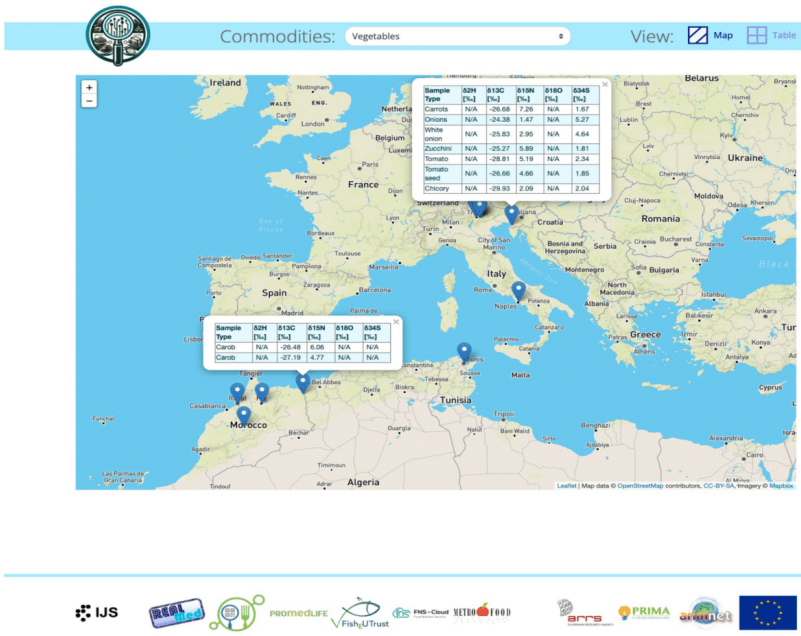


FIGURE 4
Interactive world map with the data.

Further, the metadata was selected from the ISO-FOOD ontology (13), which describes isotopic measurements with all of the necessary information required for future analysis. The ontology is linked with standard ontologies, such as Units of Measurement, Food, Nutrient and Bibliographic Ontologies.

From a higher-level perspective, the system framework and communication between the web application and its backend processes is presented in Figure 5.

Backend:

- PostgreSQL Database: the central storage of the system, containing the collected and structured data.
- Django (Data Processing): acts as the application layer for data processing and logic implementation, bridging the database and the next layer.
- Node.js Server: serves as the middleware or API server, facilitating communication between the backend and the frontend.

Frontend:

- Bootstrap: framework for IsoFoodTrack views.
- Leafletjs: API that serves map view.
- Jquery: a javascript library.

5 Application and validation

Validation of the IsoFoodTrack database was an essential step to ensure its practical application in food authentication and fraud detection. The validation process involved several stages:

5.1 Reference dataset validation

In order to reliably determine authenticity, the isotopic data of the test samples must be compared with the databank. The most straightforward and still the most recognized approach is that of univariate data evaluation, based on the arithmetic mean, median, standard deviation and authenticity limits considering the Student's t-distribution. These metrics enable users to understand the degree of heterogeneity within a given region and enhance the reliability of origin verification. A 95% confidence level is considered appropriate for commercial samples, which are produced in large batches and should have stable isotope values close to the mean values of the authentic materials. The test result can be clear, in terms of true or false, but also suspicious or unlikely, for example, when the reference databank is not robust enough to be considered reliable. The most efficient approach is to create yearly databases, particularly for vegetable and fruit commodities that exhibit significant variability in harvest and production from year to year.

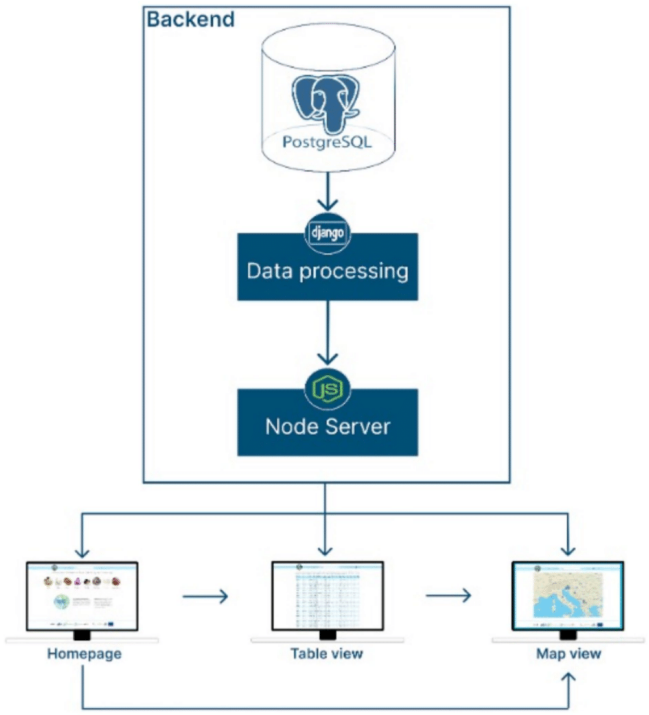


FIGURE 5
System architecture of the IsoFoodTrack framework.

Users should primarily rely on aggregated regional reference values for comparison to account for inherent variability within the dataset. In borderline cases, additional analyses such as examining secondary isotopes or incorporating external metadata (e.g., trace elements or supply chain information) are recommended. For samples classified as "suspicious," users are advised to conduct further investigations, including re-analysis or consultation with experts.

5.2 Statistical analysis

Chemometric methods or multivariate data analysis help separate information from noise, uncover hidden correlations, and visually represent them. There are three main approaches: (1) explorative analysis, (2) classification, and (3) calibration. The choice of method depends on the problem and experimental data (14). For example, principal component analysis (PCA) is commonly used initially for dimensionality reduction, highlighting the most representative features with minimal information loss and generating new variables called principal components (15). However, PCA does not consider group membership, so chemometric methods are used for classification and class modeling when focusing on product origin. Classification, synonymous with discriminant methods, assigns objects to predefined classes using techniques like linear discriminant analysis (LDA), k-nearest neighbors, partial least squares-discriminant analysis (PLS-DA), and artificial neural networks (ANN) (16).

Linear discriminant analysis, one of the simplest classifiers, requires a sufficient ratio ($\geq$3) between samples and variables and struggles with highly collinear data common in chemistry (16). PLS-DA addresses these issues, creating a linear model statistically equivalent to LDA's solution but overcoming minimum sample-to-variable ratio and collinearity problems (16). Orthogonal partial least squares-discriminant analysis (OPLS-DA), a modification of PLS-DA, enhances interpretability by separating predictive variance from non-predictive variance (17). Model performance is evaluated by explained variation (R2X for PCA and R2Y for OPLS-DA) and predictive ability (Q2), with internal sevenfold cross-validation minimizing overfitting. OPLS-DA prediction performance is measured by sensitivity (true positives) and specificity (true negatives) (18). Discriminant markers are selected by Variable Importance in the Projection (VIP) values, with a threshold of one.

Class modeling, rather than discriminant analysis, is often recommended to confirm a sample's regional origin due to possible

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biases in one-class classification problems. Soft independent modeling of class analogy (SIMCA) is a standard method in chemometrics for such tasks (19, 20).

5.3 Cross-validation

A cross-validation approach was used to evaluate the robustness of the IsoFoodTrack database. This involved splitting the dataset into training and testing sets, where the training set was used to build a predictive model, and the testing set was used to evaluate the accuracy of the predictions. High predictive accuracy indicated the effectiveness of the database in identifying food fraud and verifying the geographical origin of samples.

5.4 Practical applicability

Finally, the practical application of the IsoFoodTrack database was demonstrated by analyzing a set of commercial food samples and comparing their isotopic and elemental profiles against the reference dataset. The results confirmed the ability of the database to detect discrepancies in geographical origin and production claims, thereby validating its utility as a tool for ensuring food authenticity. The Slovenian studies include the use of different chemometric approaches for verifying the geographical region of different commodities. For example, we investigated the possibility of determining the geographical origin of milk and dairy products. Using linear discriminant analysis, discrimination and specification of goat, cow and sheep milk and cheese was possible (21). Moreover, the existing database of authentic Slovenian cow milk was used to verify the correct assignment of regional provenance and declaration of origin. By applying discriminant analysis, the ability to discriminate between geographic regions was only possible when data were organized by year and season as a result of different feeding regimes. Based on the data, a discrimination model was developed to differentiate milk from European milk produced in Slovenia efficiently. Slovenian milk was statistically distinguishable from all other milk, where the most important parameters were $\delta^{18}O$, Sr., K and *Ca*. Commercial samples labeled as "Slovenian milk" were confirmed and classified as being authentic (22).

Despite the fact that the Slovenian truffles shared some similar characteristics with the samples originating from other countries, differences in the element concentrations suggest that respective truffle species may respond selectively to nutrients from a specific soil type under environmental and soil conditions. Cross-validation resulted in a 77% correct classification rate for determining the geographical origin and a 74% correct classification rate for discriminating between species. The critical parameters for geographical origin discriminations were Sr., Ba, V, Pb, Ni, Cr, Ba/Ca and Sr./Ca ratios, while from stable isotopes $\delta^{18}O$ and $\delta^{13}C$ values are the most important (23).

Discriminant and class-modeling methods have also been applied to assess the geographical classification and authentication of selected fruits and vegetables, including strawberries, cherries, apples, kaki, asparagus and garlic, using stable isotopes of light elements and elemental composition of samples harvested between 2018 and 2020. A good geographical classification of Slovenian and non-Slovenian strawberries was obtained despite different production years using discriminant approaches. Class models generated by data-driven soft independent modeling of class analogy (DD-SIMCA) had high sensitivity (96–97%) and good specificity (81–91%) on a yearly basis, while a more generalized model combining total yearly data gave a lower specificity (63%) (24). Of the 33 commercially available samples (test samples) with declared Slovenian origin, 39% were from outside of Slovenia. The specificity for garlic and asparagus was found to be higher compared to strawberries, indicating that the model for these two commodities is more robust for verifying the correct labeling.

These examples highlight the potential of isotopic and elemental analysis as reliable tools for food origin authentication while demonstrating that some commodities present more significant challenges compared to others.

In addition, the IsoFoodTrack database can be used to support advanced analytics based on statistical and explainable machine-learning approaches, enabling the development of discriminant models to differentiate selected food commodities based on species using elemental and stable isotope data. Machine learning (ML) is a branch of artificial intelligence (AI) that enables systems to learn and improve from experience without being explicitly programmed. The ML component accepts the food's isotopic composition as input and predicts its geographical origin. Additionally, this approach offers explanations about which specific isotopes serve as indicators for that geographical origin, with the aim of increasing trust in AI-generated suggestions.

The metadata included in the database allow the user to enrich and complement a stable isotope reference database by a more novel approach, i.e., process-based modeling, such as isotopic mapping (isoscapes) (25). Isoscapes can be constructed to make predictive patterns and inform the likelihood of origin based on regional and localized characteristics. The basic concept of isoscapes is reflected in its name, derived from the words "isotope" and "landscape." Isoscapes visualize the distribution of isotopic ratios (typically of light elements) in space, often using Geographic Information System (GIS) technology to incorporate these ratios into geographic maps. There are two primary methods for producing isoscapes: statistical and process modeling. In statistical modeling, various geostatistical approaches, such as inverse distance weighting and kriging, are used to model the expected isotopic composition of the material in question. These methods typically require extensive databases that densely cover the area of interest. Only a few national-scale isoscapes studies such as wine (26), milk (27), olive oil (28), and rice (29) have been published.

Conversely, process modeling involves obtaining variables with higher spatial density, such as meteorological or geological data, to model the isotopic composition based on the processes affecting isotopic fractionation. For example, food isoscapes are derived from the observation that food produced in a specific area often reflects the local climatic and geological characteristics. The advantage of process-based modeling over statistical modeling is that it requires a much smaller sample database and can be applied to areas with limited sampling. A good example of the spatial variability "GIS modeling Isoscapes" of oxygen and carbon stable isotope composition ($\delta^{13}C$, $\delta^{18}O$) of argan oil is also presented by Taous et al. (30). In order to make global, spatially continuous predictions for argan oil stable isotope ratios, the mechanistic models in ArcGIS software (ESRI Corporation ArcGIS 10.5) were implemented. The ordinary point kriging was used to spatially interpolate $\delta^{13}C$ and $\delta^{18}O$ values of argan oils from 25 individual samples collected at five independent regions.

These geospatial models – isoscapes may provide a cost-effective extension to the isotopic dataset approach.

6 Database curation and availability

The IsoFoodTrack database (see text footnote 2) curates isotope data from authentic food samples, primarily from Slovenia and other countries, as part of various projects. For instance, in the REALMED project, data includes Moroccan argan oil, Tunisian lamb, and truffles from Italy and Slovenia, while in FishEUTrust, data on sea bream from Malta, Portugal, and Spain is included.

Future upgrades will expand the database to cover additional commodities like honey, nuts, wheat, and cereals. In addition, we also intend to supplement our data with relevant, available open-access data from the literature. Open-access data will also supplement existing datasets such as FoodIntegrity, FNS-Cloud, and METROFOOD-RI, and norms for data-sharing, including embargo periods before public release, will be established. Additionally, it complies with the ISO-FOOD ontology (13), supporting semi-automated integration with data from other relevant sources, and is openly available through the NCBO BioPortal. The nomenclature of the elemental components complies with the CEN Standard of food data.

With new data and new methods for data analysis being integrated into IsoFoodTrack, both the tool and the ontology will enable interoperability with other platforms. The underlying concept of interlinking a dataset with semantic resources serves as an excellent example of open science e-infrastructures that will need to be developed in the future.

7 Conclusion

IsoFoodTrack represents one of the initial efforts to compile stable isotope and elemental data for food authenticity and fraud detection. Its robust, scalable, and flexible architecture makes it an invaluable resource for researchers, food control agencies, and global food authenticity initiatives. Here are the key achievements and contributions of IsoFoodTrack:

Comprehensive data management: The database integrates isotopic and elemental composition data with rich metadata, encompassing geographical, production, and methodological details.

Flexibility and scalability: IsoFoodTrack is designed for continuous expansion, allowing the inclusion of new food commodities, analytical methods, and metadata fields. This ensures the database remains adaptable to ongoing research and technological developments, making it future-proof.

Interoperability and integration: The use of standardized formats like JSON and CSV, along with the potential for API integration, enables IsoFoodTrack to exchange data seamlessly with other databases such as future IsoBank. This promotes collaboration and data sharing across global research initiatives.

User-friendly access and data visualization: IsoFoodTrack's web interface, developed using Django, provides an intuitive platform for users to access, query, and interact with the data. Features like interactive maps and tabular displays allow for easy visualization and comparison of data across different geographical locations and samples, enhancing user engagement and analytical capacity.

Rigorous quality control: The database implements a thorough validation process to ensure high-quality and reliable data. All samples undergo standardized treatment and analysis, with expert review ensuring that only accurate and validated data is included. This robust approach to quality control enhances the credibility of IsoFoodTrack in scientific research.

Practical applicability: Based on the data collected in IsoFoodTrack using statistical, chemometric and machine learning approaches the database has a capability to identify and classify the origin of food commodities. It also supports isoscapes, which visualize isotopic ratios across regions, providing spatially continuous predictions of geographical origins of food products. This novel approach adds depth to isotopic analysis and extends the database's predictive capabilities, particularly in regions with limited sampling.

Future enhancements: Future upgrades include expanding the database to cover more food commodities (e.g., honey, nuts, cereals) and integrating open-access data from literature. These enhancements ensure that IsoFoodTrack will continue to evolve as a critical resource for food authenticity research.

Data availability statement

The raw data supporting the conclusions of this article are available from the corresponding author upon reasonable request.

Author contributions

CT: Conceptualization, Data curation, Investigation, Writing – original draft. RM: Data curation, Methodology, Visualization, Writing – review & editing. MO: Data curation, Methodology, Visualization, Writing – review & editing. AS: Methodology, Visualization, Writing – review & editing. JD: Methodology, Writing – review & editing. TE: Methodology, Visualization, Writing – review & editing. BKS: Methodology, Supervision, Validation, Writing – review & editing. NO: Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2025.1516521/full#supplementary-material

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3.2 Scientific Paper: "Stable Isotope and Multi-Elemental Analysis Combined with Statistical Modeling for Saffron Origin Authentication"

In this chapter, I present the paper titled "Stable Isotope and Multi-Elemental Analysis Combined with Statistical Modeling for Saffron Origin Authentication" by Cathrine Terro, Lidija Strojnik, Doris Potočnik, Marta Jagodic Hudobivnik, Darja Mazej, Luana Bontempo, Mahmoud Eddabdouby, Nour Eddine Amenzou, Benmansour Moncef, Simon Kelly, Nives Ogrinc. The article was published in Food Chemistry: X, Volume 31, in 2025.

The paper describes the integration of stable isotope ratio analysis (δ^{2}H, δ^{13}C, δ^{15}N, δ^{18}O, δ^{34}S) with multi-elemental profiling and chemometric modeling to distinguish saffron from several countries: Morocco, Greece, Slovenia, Spain, Iran, and the UK. This approach addresses gaps in previous research, which mostly used either elemental or isotopic data alone, with a few exceptions (Wakefield et al., 2019; Perini et al., 2020). Moreover, many studies have focused on limited regions, often excluding major producers like Morocco. A total of 75 saffron samples, including 48 verified authentic specimens, were analyzed using isotope ratio mass spectrometry (IRMS) and inductively coupled plasma mass spectrometry (ICP-MS). The paper incorporates two previously published datasets to create an aggregated dataset of 163 authentic samples, enabling a robust analysis of both temporal and geographic variability.

Using orthogonal partial least squares discriminant analysis (OPLS-DA), we achieved a classification accuracy of 94%, identifying δ^{2}H, δ^{15}N, K, Ca, δ^{13}C, Sr, Mn, Co, and Zn as key variables differentiating saffron origin. The inclusion of elemental ratios such as Rb/Sr, K/Rb, and Ca/Sr improved the separation of Moroccan, Iranian, and Italian samples. Further, class-specific modeling using Data-Driven Soft Independent Modeling of Class Analogy (DD-SIMCA) validated the high specificity and sensitivity of our approach, revealing that approximately 15% of commercial samples were mislabeled, with Iranian-labeled saffron showing the highest discrepancy. These findings demonstrate the strong potential of combining stable isotope and multi-elemental analyses with advanced chemometrics to enhance saffron traceability and combat fraud in this economically important spice trade.

In this study, I was responsible for acquiring a subset of saffron samples, preparing samples, and performing data curation, processing, and database development. I also wrote the initial draft of the manuscript. This work was presented as an oral presentation at the International Symposium on Food Safety and Control, held in May 2024, at the IAEA Headquarters in Vienna, Austria, and during the virtual "Spotlight on Food Fraud Talks," organized by the Food Authenticity Network, in June 2025. Additionally, the research was showcased as a poster at the 17th Jožef Stefan International Postgraduate School Students' Conference in Kamnik, Slovenia, in May 2025. This work was also presented during the FoodTraNet Project meeting and Winter School, held at Aristotle University of

Thessaloniki (AUTh) in Thessaloniki, Greece, in March 2024, and at the first FoodTraNet conference in Portorož, Slovenia, in October 2024.

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Stable isotope and multi-elemental analysis combined with statistical modeling for saffron origin authentication

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ABSTRACT

Saffron (*Crocus sativus* L.) is among the world's most valuable spices, yet its high market price and complex supply chains make it particularly vulnerable to mislabeling and fraud. To address this, we integrated stable isotope ratio analysis (δ^2H, δ^{13}C, δ^{15}N, δ^{18}O, δ^{34}S) with multi-elemental profiling and advanced statistical modeling to authenticate saffron geographical origin. A total of 75 saffron samples—48 authentic—sourced from Morocco, Greece, Slovenia, Spain, Iran, and the United Kingdom were analyzed using isotope ratio mass spectrometry (IRMS) and inductively coupled plasma mass spectrometry (ICP-MS). These were combined with two previously published datasets, including 163 authentic samples in total. The incorporation of external datasets was possible due to their strong methodological and analytical comparability with the present study, ensuring directly compatible results. Orthogonal partial least squares discriminant analysis (OPLS-DA) achieved 94 % classification accuracy, identifying δ^2H, δ^{15}N, K, Ca, δ^{13}C, Sr, Mn, Co, and Zn as key variables for discriminating geographical origins. Incorporation of elemental ratios (Rb/Sr, K/Rb, Ca/Sr) and/or δ^{18}O and δ^{34}S further refined classification, particularly improving accuracy for Moroccan saffron. Class-modeling via Data-Driven Soft Independent Modeling of Class Analogy (DD-SIMCA) confirmed high sensitivity and specificity across target sets and revealed potential mislabeling in ~15 % of commercial samples, with the highest discrepancy (24 %) observed in Iranian-labeled products. These findings underscore the power of combined stable isotope and multi-elemental approaches for saffron origin authentication and highlight the need for robust analytical verification to strengthen transparency and combat fraud in the saffron trade.

1. Introduction

Saffron, derived from the dried stigmas of *Crocus sativus*, is one of the most valuable and sought-after spices in the world. Known as "red gold," it is prized for its vibrant color, unique aroma, and versatility in culinary, medicinal, and industrial applications, including textiles, dyes, and pharmaceuticals. Its production dates back to ancient civilizations, including the Egyptians, Greeks, and Romans, underscoring its enduring cultural and economic importance (Mousavi & Bathaie, 2011; Mzabri et al., 2019). Over the centuries, its cultivation has flourished in regions such as Iran, India (Kashmir), Greece, and Spain, which are renowned for producing some of the world's finest saffron (Gresta et al., 2009; Mousavi & Bathaie, 2011). Iran, in particular, remains the largest global producer, supplying approximately 80 % of the world's saffron (Menia et al., 2018). Saffron's economic and cultural significance is deeply tied to its geographic origin, which impacts quality, consumer trust, and market value.

The production of saffron remains highly labor-intensive, requiring nearly 400 h to yield just 0.5 g of dried stigmas from 75 to 100 flowers (Cardone et al., 2019). This intensive hand cultivation contributes to its scarcity and high market value. Its unparalleled aesthetic and organoleptic qualities are attributed to bioactive compounds such as crocin,

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safranal, and picrocrocin, which contribute to its distinct flavour and health benefits, including antioxidant, anticancer, and anti-inflammatory effects (Shahi et al., 2016; Moradi Rikabad et al., 2019; Mykhailenko et al., 2020).

Local conditions contribute to the distinctive chemical profiles of saffron, which are often considered key markers of quality (Gresta et al., 2009; Mousavi & Bathaie, 2011; Mykhailenko et al., 2020). Optimal cultivation occurs in loose, well-irrigated, and effectively drained clayey-calcareous soils, further emphasizing the importance of geographic and environmental suitability (Zarghani et al., 2016). Changes in temperature, precipitation, and other climatic variables can significantly impact production, as evidenced by studies in the Kashmir Valley showing that rising temperatures and altered precipitation patterns have led to a decline in saffron yields over recent decades (Ayoub et al., 2024). Beyond climate, factors such as altitude, soil composition, water availability, photoperiod, and fertilization practices are equally critical for influencing saffron's growth and chemical composition (Cardone et al., 2020; Rahimi et al., 2017; Siracusa et al., 2010). Although its sensitivity to environmental conditions has limited its cultivation (Cardone et al., 2019), saffron production has expanded to other countries, including Italy, Turkey, France, Switzerland, Pakistan, China, Japan, and Australia. This global expansion reflects both the rising demand for saffron and the ability of cultivation practices to adapt to diverse climates and environments.

Saffron's high economic value also makes it vulnerable to various types of fraud, including mislabeling, dilution, and substitution. As global trade grows, fraudulent practices threaten the reputation of authentic producers and devalue traditional saffron-growing regions, making reliable geographic origin verification essential for ensuring authenticity, protecting market value, and preserving the cultural and economic heritage of these areas (Gresta et al., 2009). Techniques like genetic markers, sensory analysis, and chemical profiling are commonly used to authenticate saffron, each providing distinct insights. For example, dilution, where pure saffron is mixed with other substances such as paprika or turmeric (botanical adulterants), can be detected through chromatographic and spectroscopic methods that analyze the chemical composition and purity of the product (Ryparova Kvirencova et al., 2023). Substitution, involving the sale of different materials like turmeric, safflower, paprika, or marigold flowers, can be uncovered using DNA-based methods and chemical fingerprinting (Bhooma et al., 2023). The enhancement of saffron's color using synthetic dyes such as Sudan I-IV and Rhodamine B, which are potentially genotoxic, can also be detected through high-performance liquid chromatography (HPLC) and mass spectrometry (Bhooma et al., 2020; Koocheki & Milani, 2020).

Mislabeling, where the origin of saffron is falsely claimed, can be identified using stable isotopic and multi-elemental analysis, which relies on the sophisticated and widely used stable isotope approach by examining the isotopic composition of the light elements $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, and $^{34}S/^{32}S$ (Salehi et al., 2022). By analyzing these isotopic ratios, unique geographical and environmental fingerprints for each product can be created due to factors that can affect their isotopic abundance through fractionation, such as origin, climatic conditions, soil properties, and the geology of the regions where food ingredients are grown (Kelly et al., 2005).

When combined with elemental analysis, verification becomes even more powerful. The availability of elements depends on various factors, including soil pH, moisture, clay and other characteristics (Katerinopoulou et al., 2020), such as water availability and climate, while elements such as Sr, Ba, Cs, S, Mo, and Ni are related to geology (Saaltink et al., 2014). Strontium is already widely used to trace the geographical origin of agricultural produce (Hiraoka et al., 2016). Equally, Rb and Cs can be quickly mobilized in the soil and taken up by plants, making them suitable source markers (Kelly et al., 2005). The content of Na is also related to the distance to the sea, and elevated amounts can be found within several kilometres of the coast due to the steady input of marine aerosols (Saaltink et al., 2014).

Phosphorus levels in food products are often influenced by the application of phosphoric fertilizers and agrochemicals. While earlier work (Smith, 2005) suggested that fertilization has a negligible effect on elemental composition relevant to origin prediction, more recent studies challenge this assumption. For instance, a traceability study on highland barley (*Hordeum vulgare* L.) grown in Tibet demonstrated that fertilizer treatments (urea and diamine phosphate) significantly affected the concentrations of key mineral elements, including phosphorus (P), potassium (K), iron (Fe), and copper (Cu), which contributed to improved geographic classification accuracy (Zhao et al., 2022). Furthermore, elevated concentrations of certain elements such as arsenic (As), cadmium (Cd), and copper (Cu) may also result from the intensive use of fertilizers and pesticides, particularly in regions with intensive agricultural practices (Skordas et al., 2013). This highlights the importance of critically evaluating which elements are suitable for use as geographic markers, as some may reflect anthropogenic inputs rather than environmental or geological signatures.

The combination of stable isotope and elemental analysis has been successfully applied to a variety of high-value food products, such as honey (Bontempo et al., 2017; Wu et al., 2015), olive oil (Camin et al., 2010), paprika and cinnamon (Brunner et al., 2010; Primožič et al., 2025), apple juice (Bizjak Bat et al., 2016), strawberries (Strojnik et al., 2022), seafood and shellfish (Luo et al., 2019; Ni et al., 2022), beef (Bai et al., 2025), and lamb (Liu et al., 2021). In addition, it has been applied to rice, garlic, and Chinese medicinal materials (Giannioti et al., 2024; Opatić et al., 2017; Zhang et al., 2022).

Despite their proven utility, limited research has been conducted on the application of these analytical methods for authenticating the geographical origin of saffron (Maggi et al., 2011; D'Archivio et al., 2014; D'Archivio et al., 2019; Rocchi et al., 2019; Wakefield et al., 2019; Perini et al., 2020; Ghiasi & Parastar, 2021; Ibourki et al., 2022; Moras et al., 2022; Nie et al., 2023). Most focus on either elemental analysis or isotopic analysis, rarely integrating both. Notable exceptions include Perini et al. (2020) and Wakefield et al. (2019). Furthermore, studies do not fully utilize all five isotopic ratios ($\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, and $\delta^{34}S$), which are essential for capturing the complexity of saffron's chemical and isotopic profiles. Studies also tend to focus on one or two countries, with Morocco, a major saffron producer, frequently underrepresented in favor of Iran, Greece, Spain, and Italy.

This study analyzes authentic saffron samples from producers in Iran, Greece, the United Kingdom, Slovenia, Spain, and Morocco, with a particularly strong representation of Moroccan saffron. It also analyzes commercial samples from Iran, Greece, Spain, Italy, India, and Afghanistan, as well as integrating data from Perini et al. (2020) and Wakefield et al. (2019). Using this expanded dataset, advanced statistical techniques, including Data-Driven Soft Independent Modeling of Class Analogies (DD-SIMCA), were applied to verify the origin of both commercial and authentic saffron samples. By combining the available datasets, we aim to demonstrate that isotopic and elemental data are sufficiently robust and standardized to allow integration across studies, thereby strengthening the statistical power of the analysis and underscoring their potential for broader applications in future research.

2. Materials and methods

2.1. Sample collection

In total, 75 samples were included in this study (Table S1). Samples were collected in three sets: (1) samples collected directly from the producers to provide authentic samples, including from Morocco (n = 41), Greece (n = 3), United Kingdom (n = 1), Slovenia (n = 1), Spain (n = 1), and Iran (n = 1); (2) 25 samples of unverified origin, purchased from local supermarkets in Belgium (n = 3), Slovenia (n = 2), Greece (n = 6), Spain (n = 7), Italy (n = 5), and The Netherlands (n = 2), and (3) commercial saffron samples (n = 2) sourced online. The samples weighed between 0.05 g and 1 g and were either in filaments or powder

form. Dry samples were further homogenized using a ceramic mortar and pestle and stored at room temperature until analysis.

2.2. Data set compilation

Data from Perini et al. (2020) and Wakefield et al. (2019) were also integrated into the database. These datasets were incorporated due to their strong methodological and analytical comparability with the present study, ensuring that the results are directly compatible. Similar to the present study, both used isotope ratio mass spectrometry (IRMS) to analyze the isotopic composition and inductively coupled plasma mass spectrometry (ICP-MS) the elemental composition of saffron from different countries and regions with a focus on distinguishing saffron by production. Additionally, both studies utilized multivariate models—such as PLS-DA, SIMCA, and LDA. Wakefield's study, which was conducted in 2010 and 2011, focused on samples from Iran and Spain, while Perini et al.'s study (2016 to 2019) expanded the geographic coverage by including samples from Italy, Iran, and Morocco.

The present study was conducted in 2023 and includes samples from Morocco, Greece, the United Kingdom, Slovenia, Spain, and Iran. The present study also includes a significant number of Moroccan samples (n = 41) collected from villages near Taliouine (n = 30) in the Taroudant region, where Perini's samples were sourced. The present study also collected samples from Rif (Zaitoune, n = 2) and Sidi Hssain (Do Agadir, Ighri, Timasinine, n = 9), which are geographically distant from Taliouine, further expanding the geographic scope.

Together, these three studies contributed a total of 163 authentic saffron samples, including those from Morocco (n = 53), Iran (n = 52), Italy (n = 44), Spain (n = 9), Greece (n = 3), the United Kingdom (n = 1), and Slovenia (n = 1). All samples were obtained directly from producers or certified cooperatives, as also reported by Perini et al. (2020) and Wakefield et al. (2019), ensuring their traceability and authenticity. This combined dataset, excluding commercial samples, forms part of the IsoFoodTrack database (Terro et al., 2025), accessible at http://isofoodtrack.ijs.si/, and will serve as the reference set for the subsequent statistical analyses aimed at evaluating the authenticity and geographic origin of saffron. The distribution of samples is uneven across countries, with the majority originating from Morocco (32.5 %), Iran (31.9 %), and Italy (27.0 %), while the United Kingdom (0.6 %), and Slovenia (0.6 %) are less represented (Fig. 1). Table 1 represents the total number of samples, their origins, the analytical techniques employed, and the isotopes and elements analyzed.

Table 1
Total number of samples, origins, analytical techniques employed, and isotopes and elements analyzed.

Study	Type, number of samples and country of origin	Year	Method	Isotopes	Elements
Wakefield et al., 2019	Authentic: 48 Country: Iran, Spain Commercial: 2 Country: Spain	2010 2011	IRMS ICP-MS	3 isotopes: δ^2H, δ^{13}C, δ^{15}N	42 elements Li, B, Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Y, Mo, Ag, Cd, Sn, Sb, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Dy, Ho, Er, Tm, Yb, Lu, Pb, U
Perini et al. 2020	Authentic: 67 Country: Italy Morocco, Iran Commercial: 9 Country: N/A	2016 2017 2018 2019	IRMS ICP-MS	5 isotopes: δ^2H, δ^{13}C, δ^{15}N, δ^{34}S, δ^{18}O	42 elements Li, Be, B, Na, Mg, Al, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Y, Zr, Nb, Mo, Ag, Cd, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Dy, Ho, Er, Hf, Re, Pb
Our study	Authentic: 48 Country: Morocco, Greece, United Kingdom, Slovenia, Spain, Iran Commercial: 27 Country: Iran, Greece, Spain, Italy, India, Afghanistan	2023	IRMS ICP-MS QQQ	5 isotopes: δ^2H, δ^{13}C, δ^{15}N, δ^{34}S, δ^{18}O	26 elements Na, Al, Mg, P, S, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Cd, Mo, Cs, Ba, Hg, and Pb



Fig. 1. Locations of authentic saffron samples (Italy, Morocco, Iran, Spain, Slovenia, United Kingdom, Greece).

2.3. Stable isotope analysis

2.3.1. Determination of $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ values in bulk saffron samples

The stable isotope ratios of carbon ($^{13}C/^{12}C$), nitrogen ($^{15}N/^{14}N$) and sulfur ($^{34}S/^{32}S$) were simultaneously determined in homogenized dry saffron samples using an IRMS equipped with a preparative module for solid samples IsoPrime100 – Vario PYRO Cube (OH/CNS Pyrolyser/Elemental Analyzer) (IsoPrime, Cheadle, Hulme, UK). Approximately 5 mg of each sample and the same amount of WO_3 were placed in a tin capsule. The capsule was then closed with tweezers and placed in the sampler of the elemental analyzer. The results for carbon, nitrogen and sulfur were normalized against the following international reference materials USGS 88 ($\delta^{13}C = -16.06 \pm 0.07$ ‰, $\delta^{15}N = 14.96 \pm 0.14$ ‰, $\delta^{34}S = 17.10 \pm 0.44$ ‰), USGS89 ($\delta^{13}C = -18.13 \pm 0.07$ ‰, $\delta^{15}N = 6.25 \pm 0.10$ ‰, $\delta^{34}S = 3.86 \pm 0.40$ ‰); USGS91 ($\delta^{13}C = -28.28 \pm 0.08$ ‰, $\delta^{15}N = 1.78 \pm 0.12$ ‰, $\delta^{34}S = -20.85 \pm 0.40$ ‰), USGS43 ($\delta^{15}N = 8.44 \pm 0.10$ ‰, $\delta^{34}S = 10.46 \pm 0.22$ ‰); and IAEA-600 ($\delta^{13}C = -27.77 \pm 0.04$ ‰, $\delta^{15}N = 1.00 \pm 0.20$ ‰). The laboratory reference material CRP-CASEIN with $\delta^{13}C = -20.3 \pm 0.09$ ‰, $\delta^{15}N = 5.62 \pm 0.19$ ‰, $\delta^{34}S = 4.18 \pm 0.74$ ‰) was used for the control. The results were expressed as mean values and were calculated from triplicate analyses. The reproducibility for $\delta^{13}C$ and $\delta^{15}N$ was ±0.2 ‰, and ± 0.7 ‰ for $\delta^{34}S$.

2.3.2. Determination of $\delta^{2}H$ & $\delta^{18}O$ of saffron samples

For determining the $^{2}H/^{1}H$ and $^{18}O/^{16}O$ ratios, about 0.2 mg (between 0.20 and 0.26 mg) of each sample was weighed directly into a silver capsule and simultaneously measured on a DELTA XP IRMS (Thermo Scientific) coupled with TC/EA pyrolyzes (Thermo Finnigan). In order to remove the exchangeable hydrogen effect, the packed reference materials and samples were left open in the laboratory for 5 days in a desiccator to reach an equilibration for exchangeable hydrogen in the organic matrix with environmental hydrogen. Each sample was measured twice, and the mean values were considered. Normalization of results was performed based on two reference materials: CBS (Caribou Hoof Standard) with $\delta^{2}H$ values of -157 ± 0.9 ‰ and $\delta^{18}O$ values of 3.8 ± 0.3 ‰, and KHS (Kudu Horn Standard) with an average $\delta^{2}H$ value of -35.3 ± 1.1 ‰ and $\delta^{18}O$ value of 20.3 ± 0.3 ‰. Measurement precision was ±0.5 ‰ for $\delta^{18}O$ and ± 1 ‰ for $\delta^{2}H$.

All isotopic ratios are reported using the δ – notation in ‰ (Eq. (1)):

$$\delta\left({}^{i/j}E\right) = \delta^{i/j}E = \frac{{}^{i/j}R_P - {}^{i/j}R_{Ref}}{{}^{i/j}R_{Ref}} \quad (1)$$

where superscripts *i* and *j* denote the highest and the lowest atomic mass number of element E, and R_P and R_{Ref} indicate the ratio between the heavier and the lighter isotope ($^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{18}O/^{16}O$, $^{15}N/^{14}N$, $^{34}S/^{32}S$) in the sample (P-product) and reference material (Ref), respectively (Brand et al., 2014). The $\delta^{2}H$ and $\delta^{18}O$ values are reported relative to the V-SMOW (Vienna-Standard Mean Ocean Water) standard, $\delta^{13}C$ values to the V-PDB (Vienna-Pee Dee Belemnite) standard, and the $\delta^{34}S$ sulfur values relative to the V-CDT (Vienna Cañon Diablo Troilite) standard. The $\delta^{15}N$ values are reported relative to AIR.

2.4. Elemental analysis

Approximately 0.15 g of the dry sample was weighed into a Teflon digestion vessel, and 1 mL of Suprapur 65 % HNO_3 (Carlo Erba) was added. The contents were then digested using the Ultrawave digestion system (Single Reaction Chamber Microwave Digestion System, Milestone, Italy). The program was as follows: heating to 240 °C in 20 min, holding for 15 min at max 100 bar at a max power of 1500 W. The digestate was diluted to 10 mL with Milli-Q water (Merck, Millipore, Watertown, MA, USA) and further diluted with 5 % HNO_3 in a ratio 1:5 and 1:10. Selected elements — sodium (Na), aluminum (Al), magnesium (Mg), phosphorus (P), sulfur (S), potassium (K), calcium (Ca), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), selenium (Se), rubidium (Rb), strontium (Sr), cadmium (Cd), molybdenum (Mo), cesium (Cs), barium (Ba), mercury (Hg), and lead (Pb) — were determined using an ICP-QQQ (Agilent 8800, Japan). Quantification was achieved using external calibration. For quality control, every tenth sample was prepared in duplicate, blank and selected reference materials (Spinach Leaves NIST 1570, Tomato Leaves NIST 1573a, Peach Leaves NIST 1547) were also prepared in the same way as the samples. Detection limits were established based on standard deviations of blank measurements, with detailed optimization described in Potočnik et al. (2021). The LoDs are presented in Table S2.

2.5. Statistical evaluation

Preliminary exploratory statistical analysis of the data was conducted using box plots to visualize and compare the distribution of isotopes and elements across different years and countries. The Kruskal-Wallis test, a non-parametric method, was used to identify statistically significant differences ($p < 0.05$) among different saffron samples using the XLSTAT software package (ADDinsoft, New York, USA). For the merged analysis, only elements that met the following criteria were retained: (i) measured in all three datasets, (ii) consistently detected above the limit of detection (LOD) in more than 30 % of total samples, and (iii) demonstrated acceptable data quality suitable for robust statistical modeling. As a result, 18 elements were included in the statistical analysis: macronutrients – magnesium (Mg), potassium (K), calcium (Ca), and sodium (Na); trace elements aluminum (Al), manganese (Mn), iron (Fe), copper (Cu), zinc (Zn), molybdenum (Mo), vanadium (V), and cobalt (Co); earth elements - rubidium (Rb), barium (Ba), strontium (Sr), and cesium (Cs); and potentially toxic elements - cadmium (Cd) and arsenic (As). Each value was then divided by the LOD and taking the natural logarithm of the result, which converts distributions with long "tails" into a more Gaussian form (Mahynski et al., 2025). We generally found the logarithm to be unnecessary during modeling, though it was initially used to remove outliers from the data.

Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) was employed to explore the relationships between predefined categories or groups within the data using SIMCA-P (version 15, Sartorius Stedim Biotech, Umeå, Sweden). The model's predictive performance was evaluated R2X(cum), R2Y(cum) and Q2(cum). Model robustness was confirmed by permutation testing ($N = 100$) and by CV-ANOVA (Fiamegos et al. (2021). Candidates for discriminant markers were selected based on the Variable Importance in Projection (VIP) values from the OPLS-DA models. All VIP values >1 were considered significant and used as the threshold for selecting the most relevant discriminant markers for distinguishing between groups. The Data-Driven Soft Independent Modeling of Class Analogy (DD-SIMCA) method was also applied to validate class membership since class modeling is more suitable than classification methods for controlling the origin or authenticity of foods (Forina et al., 2008). DD-SIMCA analyses were conducted utilizing the modified DD-SIMCA MATLAB GUI tool (Zontov et al., 2017) alongside the mdatools package in R version 4.0.3 (Kucheryavskiy, 2020) described in more detail by Strojnik et al. (2022) and Mahynski et al. (2025). Sensitivity and specificity were used as figures of merit. The sensitivity denotes a share of correctly identified samples of the target class, while specificity is a portion of objects of an alternative class correctly identified as members of that alternative class. The sensitivity can be defined as 100 (1– α)% and specificity as 100 (1– β)% (Fidelis et al., 2017; Rodionova, Oliveri, & Pomerantsev, 2016; Rodionova, Titova, & Pomerantsev, 2016). In addition to sensitivity and specificity, the DD-SIMCA models were also evaluated using total efficiency (TEFF), calculated as: TEFF = (Sens+Spec)/2.

3. Results and discussion

3.1. Exploratory statistical analysis

3.1.1. Temporal variation

Variability between production years must be considered when creating an adequate training set for the model to improve the reliability of geographic origin determination. In this study, a sufficient number of samples from different production years were only available for Morocco, Italy, and Iran. A Kruskal-Wallis test was then applied to determine statistically significant differences ($p < 0.05$) in the variables across sampling years (Table 2; Fig. S1).

The results highlight year-to-year fluctuations in environmental and agricultural conditions influencing the isotopic and elemental profiles of the samples. a broader range from −105.3 ‰ to −69.7 ‰, suggesting greater variability in environmental water sources. Statistically significant temporal patterns have been observed in δ^2H and δ^{18}O values in Italian saffron illustrating further their sensitivity to climatic conditions. For instance, the Italian saffron samples show an increase in δ^{18}O values between 2016 and 2017, coinciding with a fourfold reduction in precipitation, particularly in October, just before the harvest (Perini et al., 2020). A positive correlation was observed between δ^2H and δ^{18}O values ($\delta^2H = 3.44 \cdot \delta^{18}O - 168$ ‰; $r^2 = 0.534$), indicating the influence of biosynthetic fractionation within the plant. The weaker correlation and lower slope compared to the meteoric water line ($\delta^2H = 8 \cdot \delta^{18}O + 10$ ‰) suggest that, while plant tissues retain part of the environmental water signal, deviations arise from physiological processes. The observed correlation between δ^2H and δ^{18}O suggests that comparable isotopic approaches hold considerable potential for tracing and geolocating the origin of saffron. Further the only statistically significant difference in stable isotope analysis was determined in δ^{13}C values in Iranian samples, with mean values of −25.1 ‰ in 2016, −24.4 ‰ in 2017, −25.0 ‰ in 2018, and − 26.4 ‰ in 2023, with the more negative value observed in 2023 likely reflecting improved water availability or different climatic conditions, which reduced water stress compared to earlier years.

In Morocco, with the exception of Mg, which showed a decreasing trend over time, other elements such as P, K, Ca, and Cu show statistically significant increasing trend with production year. Other elements like Sr and Zn showed more variability but also revealed significantly higher levels in 2023. This trend can be attributed to Morocco's intensified efforts to improve soil fertility and agricultural productivity. Initiatives such as the Fertimap program, which provides tailored fertilizer recommendations based on detailed soil fertility mapping (Starling, 2023), have likely contributed to elevated levels of these elements in soils (Starling, 2023). Additionally, prolonged drought conditions may have concentrated certain minerals in soils due to reduced leaching, further influencing these observed increases (Toreti et al., 2023). Notably, δ^{15}N values in Moroccan samples decreased from 8.1 ‰ (SD = 1.02) in 2016 to 5.2 ‰ (SD = 3.71) in 2023, consistent with shifts in agricultural practices and fertilizer use in Morocco.

A similar situation was also observed in Iran, where the concentrations of elements exhibited significant variability between production years. For example, K levels initially increased, peaking before a decline in 2023, while Ca levels showed a notable peak followed by a decrease in 2023. Iron concentrations also varied over the years, while Cu showed a slight increase. Similarly, as in Moroccan saffron, levels of Zn significantly rose during the sampling period while Sr levels fluctuated. Mo and V levels both showed gradual increases, respectively. Cadmium (Cd) and arsenic (As) levels also exhibited variability. Cadmium reached its peak in 2023, while As concentrations fluctuated over the years, initially showing a sharp increase followed by moderate fluctuations in the subsequent years leading up to 2023.

3.1.2. Geographical variation

For the geographical comparison, all available countries were included to capture the broader spatial variability. The isotopic composition of δ^2H, δ^{13}C, δ^{15}N, δ^{18}O, and δ^{34}S varied across the studied regions and can be visualized in Fig. 2. To contextualize these results, a comparative summary of published stable isotope data for saffron from various geographic origins was compiled (Table S3). This table presents sample origins along with corresponding isotopic ranges, means, and standard deviations, facilitating cross-comparison of isotopic signatures across regions.

Statistically significant differences ($p < 0.05$) were observed for all variables, except for Cu ($p = 0.064$). Differences between countries in variables such as δ^2H, δ^{13}C, Na, Al, and Zn were highly significant ($p < 0.0001$), suggesting their potential utility as discriminant markers for geographical authentication. Notably p-values <0.0001 for δ^2H, δ^{13}C values and the elemental concentrations of magnesium (Mg), potassium (K), calcium (Ca), sodium (Na), aluminum (Al), manganese (Mn), iron (Fe), zinc (Zn), barium (Ba), strontium (Sr), molybdenum (Mo), vanadium (V), cobalt (Co), cadmium (Cd), arsenic (As), and cesium (Cs). These data are presented in table S4 and visualized in Fig. S2 for Italy, Morocco and Iran.

δ^2H values ranged from −105.3 ‰ (Italy) to −51.0 ‰ (Morocco), with Italian saffron exhibiting the lowest mean δ^2H (−87.8 ‰, SD: 7.2 ‰) and Moroccan samples the highest (−70.7 ‰, SD: 9.0 ‰). Notably, Maggi et al. (2011) reported substantially less negative δ^2H values for Italian saffron (−72.4 ‰), contrasting with those observed in the present study and closer to Moroccan averages. This discrepancy likely reflects regional climatic differences, as Maggi et al.'s samples originated from Sardinia, a Mediterranean island with a warmer, drier climate and isotopically enriched precipitation. In the Medio Campidano saffron-growing area of Sardinia, mean annual temperatures are ~17–18 °C and annual precipitation averages ~450–600 mm, reflecting the region's warm, semi-arid Mediterranean climate (ISPRA, 2020). In contrast, Perini et al. (2020) predominantly included samples from central Italy (L'Aquila and Spoleto), where higher altitude, cooler mean annual temperatures (~10–12.5 °C), and moderate annual precipitation (~ 700–900 mm) contribute to more depleted δ^{2}H values (−87.8 ‰)

Table 2
Overview of isotope and elemental markers with statistically significant differences in saffron samples from Italy, Morocco, and Iran across different years. Asterisks indicate the level of statistical significance (*p < 0.05; **p < 0.01; ***p < 0.001) for each variable between geographic origins. Blank cells indicate non-significant differences.

Variable	Italy (2016–2017)	Morocco (2016–2018–2019–2023)	Iran (2016–2017–2018-2023)
δ^2H [‰]	*		
δ^{18}O [‰]	***	***	
δ^{34}S [‰]			
δ^{13}C [‰]			*
δ^{15}N [‰]			
Mg [mg/g]		**	
K [mg/g]		***	**
Ca [mg/g]		**	**
Na [μg/g]			
Al [μg/g]			
Mn [μg/g]			
Fe [μg/g]			*
Cu [μg/g]		**	**
Zn [μg/g]		***	***
Rb [μg/g]			*
Ba [μg/g]			
Sr [μg/g]		*	*
Mo [μg/g]			*
V [ng/g]			*
Co [ng/g]			
Cd [ng/g]			*
As [ng/g]			*
Cs [ng/g]			

Note: *p < 0.05 = statistically significant; ** p < 0.01 = highly significant; ***p < 0.001 = very highly significant.

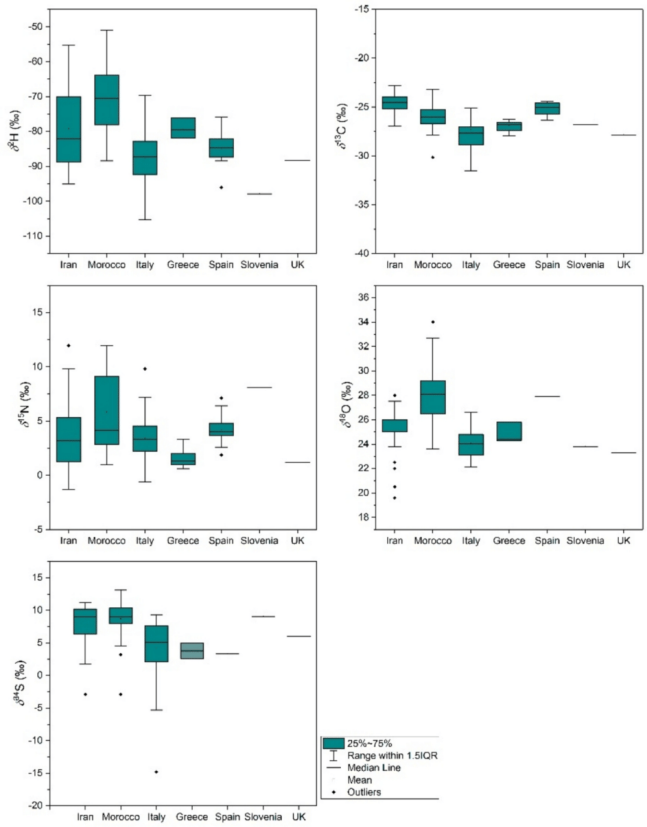


Fig. 2. Box plots of δ^2H, δ^{13}C, δ^{15}N, δ^{18}O, δ^{34}S values in saffron samples from Iran, Morocco, Italy, Greece, Spain, Slovenia, and the United Kingdom.

(Climate-Data.org, 2025). These findings underscore the sensitivity of δ^2H to local agroclimatic conditions and demonstrate the power of stable isotope analysis in discriminating not only between countries, but also between subregional origins within the same country.

Spain showed the highest δ^{18}O value in our dataset (27.9 ‰), however, this is based on a single sample ($n = 1$) and therefore cannot be interpreted as a population estimate. Thus, Morocco exhibits the highest country-level mean (27.6 ‰, SD: 2.3 ‰), followed by Iran (25.8 ‰, SD: 1.2 ‰), Greece (24.8 ‰, SD: 0.8 ‰), and Italy (24.1 ‰, SD: 1.2 ‰). This gradient is consistent with climatic expectations, with higher δ^{18}O values in the warmer, more evaporative environment of Morocco (mean annual temperature; 16.7 °C; ~228 mm annual precipitation in the Taliouine region) and lower values in cooler, more humid regions such as Italy and Greece (Climate-Data.org, 2025). For comparison, Nie et al. (2023) reported Iranian saffron δ^{18}O values in close agreement with our Iranian results (25.2 ‰), suggesting that δ^{18}O for Iranian saffron remains relatively stable across seasons and years, likely reflecting consistent cultivation conditions in the main producing regions. In the same study, Chinese saffron showed a mean δ^{18}O of 24.9 ‰ (SD: 1.1 ‰), similar to values reported for Mediterranean growing regions such as Greece (24.8 ‰), where the saffron-producing region of Kozani is characterized by a mean annual temperature of ~12.4 °C and mean annual precipitation of ~568 mm (Hellenic National Meteorological Service, 2025). However, the Chinese samples originated from contrasting production areas, with humid eastern provinces and high-altitude Tibet (>4000 m) expected to yield more negative δ^{18}O values, while semi-arid central and southwestern regions would tend to have higher δ^{18}O values. This averaging effect results in a national mean that coincidentally aligns with Mediterranean levels (Nie et al., 2023). These findings highlight the importance of considering subnational variation when interpreting isotopic data, since δ^{18}O values are shaped by both regional climate conditions and cultivation environments. Consequently, δ^{18}O should be assessed at appropriate spatial scales rather than relying solely on national averages.

δ^{13}C values ranged from −30.2 ‰ in Morocco (mean: −26.0 ‰) to −22.8 ‰ in Iran (mean: −24.7 ‰). The relatively low mean δ^{13}C value for Italian saffron (−27.7 ‰) is consistent with C_3 plant metabolism under cooler, nutrient-rich conditions. By contrast, the higher δ^{13}C values observed in Iranian saffron likely reflect the combined effects of arid conditions and water stress, linked to reduce groundwater recharge during the growth period (Noori et al., 2023). In Mashhad, Iran's primary saffron-producing region, the rainless season extends for about

nine months (April to February), with August receiving on average only ~1 mm of precipitation. These dry conditions coincide with extreme summer heat, with peak daily temperatures in July reaching ~35 °C (WeatherSpark, 2025). These findings are broadly consistent with several previous studies. For instance, Maggi et al. (2011) reported nearly identical $\delta^{13}C$ values for Iranian saffron (mean: −24.5 ‰), which are further corroborated by Ghiasi & Parastar (2021) (mean: −24.6 ‰) and Nie et al. (2023) (mean: −25.0 ‰), highlighting the stability of carbon isotope signatures under Iran's prevailing agroclimatic conditions. For Italy, however, Maggi et al. (2011) reported slightly less negative $\delta^{13}C$ values (−26.6 ‰), most likely reflecting the warmer and drier conditions of their sampling locations. Rocchi et al. (2019) reported an intermediate mean $\delta^{13}C$ value of −26.6 ‰ for Italian saffron, but with a notably large standard deviation (±11.2 ‰), reflecting the diverse origins of their samples across both northern and central mainland regions (e.g., Abruzzo, Lombardy) as well as southern areas such as Sardinia. This wide variability underscores the influence of local agroclimatic conditions on carbon isotope ratios and reinforces the discriminatory power of $\delta^{13}C$ for tracing subregional origin.

$\delta^{15}N$ values ranged from 2.3 ‰ (Greece) to 5.8 ‰ (Morocco), with Moroccan saffron exhibiting the highest mean (5.8 ‰, SD: 3.5 ‰) and Greek samples the lowest (2.3 ‰, SD: 1.2 ‰). Higher $\delta^{15}N$ values in Moroccan saffron are consistent with the predominant use of organic fertilizers, particularly manure, which becomes enriched in ^{15}N through microbial mineralization and decomposition (Aziz & Sadok, 2015; Bateman et al., 2007; Vitòria et al., 2004). This practice aligns with Moroccan saffron cultivation soils characterized by coarse texture, low organic matter (<1 %), alkaline pH, and well-draining conditions typical of semi-arid zones, where manure addition is crucial to improve soil fertility and organic content (El Grah et al., 2022). In contrast, lower $\delta^{15}N$ values in Greek saffron align with Maggi et al. (2011), who reported even lower values (1.2 ‰, SD: 0.4 ‰), reflecting predominant use of synthetic nitrogen fertilizers or low-input systems relying mostly on natural nitrogen cycling. Greek saffron is typically cultivated in Mediterranean climates with hot, dry summers and mild, wet winters, characterized by average annual temperatures around 15 °C to 20 °C and annual precipitation of approximately 500 mm, mostly concentrated in spring, which is beneficial before flowering (Rocosaffron, 2025; Saffron Business, 2020). Such climatic conditions combined with well-drained soils reduce the need for organic amendments compared to Morocco. Spanish saffron exhibited intermediate $\delta^{15}N$ values (4.1 ‰, SD: 1.7 ‰), consistent with previous reports (Maggi et al., 2011), and similarly indicative of organic fertilizer use. Iranian saffron (3.7 ‰, SD: 1.1 ‰) closely matched literature values (Nie et al., 2023) and is consistent with the systematic application of synthetic nitrogen fertilizers in conjunction with clayey to sandy loam soils of low organic matter content (<1 %), typical of Iranian cultivation areas (Kafi et al., 2018; Mykhailenko et al., 2020). Italy displayed a wider $\delta^{15}N$ range, likely reflecting mixed fertilization practices, alternating between synthetic and organic inputs.

The sulfur isotopic composition of saffron primarily reflects the $\delta^{34}S$ signature of the soils and fertilizers used in cultivation. Regional geology strongly affects soil sulfur, as bedrock sulfates and sulfides vary widely in isotopic composition. Proximity to the coast can also play a role, since marine aerosols are typically enriched in ^{34}S compared with terrestrial sulfate. Agricultural practices contribute further variability: manure and organic fertilizers often show higher $\delta^{34}S$ values, while synthetic fertilizers tend to have lower $\delta^{34}S$ values. Biological fractionation in plants is generally small (−4 to +0.5 ‰ during assimilatory sulfate reduction), meaning that saffron largely records the isotopic composition of its sulfur source. Consequently, $\delta^{34}S$ values in saffron can serve as tracers of both regional geochemical background and cultivation practices. Morocco showed the highest country-level mean among regions with multiple samples (8.6 ‰, SD: 2.7 ‰), followed by Iran (7.6 ‰, SD: 4.4 ‰) and Italy (4.2 ‰, SD: 4.8 ‰), Spain (3.3 ‰), Slovenia (9.1 ‰), and the UK (6.0 ‰). Higher $\delta^{34}S$ in Moroccan saffron may reflect the combined influence of coastal marine sulfate deposition and/or manure-based fertilization and, while the lower values in Greece (3.8 ‰, SD: 1.2 ‰) are consistent with greater reliance on synthetic fertilizers. As noted by Perini et al. (2020), the isotopic composition of sulfur is influenced by multiple overlapping factors, making straightforward interpretation difficult despite its utility for origin discrimination. The lowest $\delta^{34}S$ value (−14.8 ‰) was observed in the Italian sample. Similar $\delta^{34}S$ value of −15.4 ‰ has been reported in truffles from Italy, suggesting that very low $\delta^{34}S$ is not a universal characteristic but rather a region-specific marker (Hamzić Gregorčič et al., 2020). In particular, such values are typical of the Perugia region, indicating a unique geochemical signature that may also be linked to saffron production in that region.

Significant regional differences were also identified in macroelements (Mg, K, Ca) and microelements (Mo, Sr, Fe), which can largely be attributed to variation in soil composition, fertilization, and irrigation practices. Magnesium concentrations ranged from 0.770 mg/g in Italian samples to 3.98 mg/g in Iranian saffron, with Iran showing the highest mean level (1.77 mg/g, SD: 0.55 mg/g). Potassium ranged from 5.57 mg/g (Italy) to 33.5 mg/g (Iran), with Spanish saffron exhibiting the highest mean concentration (19.9 mg/g, SD: 2.94 mg/g), followed closely by Iranian (18.7 mg/g, SD: 4.32 mg/g). Moroccan saffron displayed intermediate values (mean: 14.5 mg/g, SD: 2.18 mg/g), falling below Spanish and Iranian samples but above Italy.

These differences can be explained by agricultural practices and water management. In Italy, saffron is typically cultivated under rainfed conditions, with over 90 % of farms relying exclusively on local rainfall (Cicco et al., 2025; Giupponi et al., 2023). By contrast, saffron cultivation in Iran and Morocco involves controlled irrigation during key phenological stages, such as flowering (October) and the reproductive phase (March–April), often using groundwater or nutrient-rich water sources (Aziz & Sadok, 2015; Sepaskhah & Kamgar, 2009). Such practices directly influence mineral availability and uptake. The elevated K levels in Moroccan samples likely reflect the application of K-rich fertilizers, such as those supplied through the Khemisset Potash Project (Emmerson Plc, 2021). Similarly, Iran increased potash fertilizer use, including potassium sulfate (SOP) and Muriate of Potash (MOP), from the mid-1990s onward to improve yields (Malakouti, 2004). This trend is consistent with the observed antagonistic relationship between K^+ and Mg^{2+} in plant uptake, where increased K availability can suppress Mg absorption (Yang et al., 2024). The marked decrease in Mg concentrations in 2023 may therefore be linked to elevated potassium fertilization.

Industrial and agricultural activities also contribute to trace element patterns. Phosphate fertilizers are a recognized source of Cd and As in soils (Davenport & Peryea, 1991; Dharma-Wardana, 2018). In Greek saffron, elevated As levels may be linked to lignite mining in Western Macedonia, while vanadium enrichment in Iranian samples likely reflects oil extraction and related industrial activities (Vitòria et al., 2004). In arid regions, limited rainfall reduces leaching, leading to higher concentrations of soluble elements such as Rb in soils, which plants may then absorb more readily (Nathan, 2017).

Calcium and strontium concentrations also showed clear regional variation, reflecting underlying geology. Both elements are associated with carbonate-rich substrates, where limestone and dolomite enhance their availability through weathering (Boggs, 2006; Brand et al., 1998). Spanish saffron showed the highest mean Ca concentration (1.77 mg/g, SD: 0.803 mg/g), followed by Iranian (1.32 mg/g, SD: 0.77 mg/g). Italian samples exhibited the lowest mean Ca levels (0.287 mg/g, SD: 0.132 mg/g), while Moroccan saffron displayed intermediate values (0.883 mg/g, SD: 0.379 mg/g). Iranian saffron also had the highest Sr concentrations (mean: 11.3 μg/g, range: 1.64–28.0 μg/g), followed by Spanish (8.66 μg/g, range: 5.84–12.6 μg/g). Moroccan saffron contained the lowest mean Sr (2.12 μg/g), while Italian samples showed wide variability (0.358–59.1 μg/g; mean: 2.74 μg/g).

Other trace elements further underline environmental and agricultural influences. Italian saffron had the highest mean Mo concentration

(0.707 μg/g, SD: 0.436 μg/g), followed by Moroccan samples (0.574 μg/g, SD: 0.394 μg/g), while Greek (0.181 μg/g) and Spanish (0.304 μg/g) saffron showed lower levels. Iron concentrations varied markedly, from 30.3 μg/g in Italy to 2127 μg/g in Iran, with Iranian saffron showing the highest mean values (280 μg/g, SD: 341 μg/g). Aluminum also displayed strong regional contrasts, with Iranian samples ranging from 38.3 μg/g to 1758 μg/g (mean: 234 μg/g, SD: 297 μg/g), compared to consistently low values in Italy (mean: 31.0 μg/g, SD: 27.3 μg/g).

These findings align with earlier studies, such as those by D'Archivio et al. (2019), who highlighted elements like Sr, Ca, Mo, and Fe as key discriminants of saffron samples from Iran and Italy. The high variability of Fe and Al in our dataset further supports their role in distinguishing samples from diverse origins, particularly between saffron from Iran and Morocco. Ibourki et al. (2022) highlighted K as the dominant macroelement and Fe as the primary microelement in Moroccan agricultural products, reporting minimal variation in K concentrations across saffron samples. The results of the present study support this observation, as the standard deviation of K in Moroccan samples was 2.18 mg/g, indicating only moderate variability. The narrow range of K concentrations (10.2–18.9 mg/g) further supports the consistency of K levels, which likely reflects uniform soil conditions or agricultural practices in the sampled regions. This consistency with previous findings reinforces the geographical significance of these elements in agricultural products.

While the absolute concentrations of elements such as rubidium (Rb), strontium (Sr), potassium (K), and calcium (Ca) may change over time due to environmental or agricultural factors, their ratios (e.g., Rb/Sr, K/Rb, and Ca/Sr) provided more stable and reliable markers for geographical differentiation being less sensitive to temporal changes. The K/Rb ratio varies significantly across different rock and soil types, supporting its use in geographical separation. The Rb/Sr ratio helps trace environmental changes and geological events, such as volcanic activity, erosion, or sedimentation, which redistribute Rb and Sr in soils and rocks, leaving distinct geochemical markers (Nebel, 2014). Also, Ca/Sr has long been used as a chemical tracer in geochemistry and hydro-geochemistry (Land et al., 2000; Pett-Ridge et al., 2009), further validating its role in geographical differentiation. The variation in Rb/Sr, Ca/Sr, and K/Rb ratios between different countries provided valuable markers for regional differentiation (Fig. S3). Iranian saffron exhibits the highest and most variable K/Rb ratios, which are statistically significantly different from those of Italian and Moroccan samples. Italian saffron consistently shows higher Rb/Sr ratios, providing a strong marker to differentiate it from Iranian and Moroccan origins. The Ca/Sr ratio also proves effective for country-level separation despite fluctuations in absolute Ca and Sr concentrations. Moroccan saffron displays statistically significantly higher Ca/Sr values than Italian and Iranian samples, likely reflecting differences in carbonate content and weathering processes.

To further investigate the discriminatory potential of these variables, pairwise comparisons between the seven countries were conducted, focusing on the significantly different variables. These comparisons revealed distinct patterns in isotopic and elemental profiles that enabled differentiation between specific country pairs. The complete pairwise comparison results are provided in Table S5. For example, $\delta^{13}C$ and Sr clearly distinguished saffron from Greece and Iran, while $\delta^{2}H$, $\delta^{13}C$, and Mg were effective in differentiating saffron from Iran from Italy. Notably, Italian and Moroccan samples displayed significant differences across a broad range of elements, including $\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, Mg, K, and Sr, among others. Samples from Slovenia and the United Kingdom ($n = 1$ each) showed no significant differences in pairwise comparisons, likely due to insufficient sample sizes and were excluded.

3.2. Orthogonal partial least squares discriminant analysis (OPLS-DA)

An OPLS-DA model was developed to differentiate saffron samples originating from Italy, Morocco, Iran, and Spain. The sufficient number of samples across the three studies provided a robust representation of geographical variation. For this differentiation, all stable isotopes were included alongside 18 elemental variables, as defined by the previously described criteria, supplemented with the Rb/Sr, K/Rb, and Ca/Sr ratios. Because the number of Spanish samples was limited, a separate OPLS-DA model was constructed to compare only Iranian and Spanish samples. This analysis achieved a clear separation between the two groups, with a classification accuracy of 97 %. The most discriminating variables were Ca/Sr, Rb, $\delta^{18}O$, Cd, Ca, and Co. For comparison, Wakefield et al. (2019) also reported a potential differentiation between Iranian and Spanish samples (from 2011) using LDA, where Ce, Al, Rb, Ba, and $\delta^{13}C$ were identified as key discriminators.

In the next step, we focused on three countries with comparable sample sizes. Because $\delta^{18}O$ and $\delta^{34}S$ were not measured in all cases, the analyses were performed using different parameter combinations. A summary of the results obtained from the various models is presented in Table 3. Models displayed moderate R^2X values (0.649–0.689), indicating that a substantial proportion of the systematic variability in the predictor data was modeled. At the same time, the models achieved high R^2Y values (0.733–0.810) and strong Q^2 values (0.698–0.771), which together confirm good model fit and predictive reliability. All OPLS-DA models were validated by permutation testing ($N = 200$) and CV-ANOVA. The permutation tests ($n = 200$) yielded R^2 intercepts ranging from 0.042 to 0.069 and Q^2 intercepts from −0.168 to −0.201 (Table 3).

These values are within accepted thresholds (R^2 intercept <0.3; Q^2 intercept <0), indicating that the models were not over fitted and that the observed discrimination is statistically significant. CV-ANOVA further confirmed model robustness, with highly significant p-values (< 0.0001) and large F values (> 38). Both models exhibited excellent classification performance, with Model M2 (incorporating Rb/Sr, K/Rb, and Ca/Sr ratios; Fig. 3) achieving 97 % overall accuracy. Notably, this model improved the correct classification of Moroccan samples from 92.4 % to 94.3 %. The inclusion of these ratios also underscored the distinctive isotopic and elemental signatures of Italian saffron, further supporting its clear separation from other regions. Among the most influential discriminators, Ca/Sr and K/Rb were particularly prominent.

Model M3, which incorporated $\delta^{18}O$ and $\delta^{34}S$ values alongside other isotopic parameters and without elemental ratios, achieved the highest classification accuracy (99 %), improving the correct classification rate for Moroccan samples to 98.1 %. The enhanced separation is primarily driven by $\delta^{18}O$, which clearly reflects water regime and climate: Moroccan saffron showed consistently higher $\delta^{18}O$ values (mean 27.6 ‰, SD 2.3 ‰) due to arid, evaporative conditions, compared with lower values in rain fed Italian saffron (mean 24.1 ‰, SD 1.2 ‰) and more stable values in irrigated Iranian saffron (mean 25.8 ‰, SD 1.2 ‰). In contrast, $\delta^{34}S$ contributed more modestly to the discrimination. Moroccan saffron tended toward higher $\delta^{34}S$ values (mean 8.6 ‰, SD 2.7 ‰), consistent with manure-based fertilization and possible marine sulfate deposition, while Iranian and Italian samples showed broader and partially overlapping ranges (means 7.6 ‰ and 4.2 ‰, respectively). Thus, $\delta^{34}S$ provides complementary but secondary information linked to nutrient sources and local geochemistry. Statistically significant differences were observed in $\delta^{18}O$ both between years and between regions, whereas $\delta^{34}S$ did not vary significantly between years but did show significant differences between countries (Table 2, Table S4). These orthogonal signals of water regime ($\delta^{18}O$) and nutrient source ($\delta^{34}S$) complement elemental markers such as K, Sr, and Ca, which may vary across years due to fertilizer application or drought. Their combined effect explains why the inclusion of $\delta^{18}O$ and $\delta^{34}S$ sharpened country-level boundaries in OPLS-DA, thereby reducing overlap and accounting for the higher classification accuracy achieved in Model M3.

3.3. Verifying labelling using class Modeling (DD-SIMCA)

Unlike discriminant methods, DD-SIMCA distinguishes objects of a particular class, i.e., the target class, from all other objects and classes,

Table 3
Summary of OPLS-DA models for discerning samples from Iran, Italy and Morocco.

Models based on	R^2X	R^2Y (cum)	Q^2 (cum)	Perm. R^2 int.	Perm. Q^2 int.	CV-ANOVA p	CV-ANOVA F	Parameters VIP > 1
M1: Stable isotopes and elemental analysis	0.661	0.733	0.698	0.042	−0.168	<0.0001	38.07	δ^2H, K, δ^{15}N, δ^{13}C, Mn, Ca, Sr, As
M2: Stable isotopes and elemental analysis with Rb/Sr, K/Rb, and Ca/Sr	0.689	0.801	0.760	0.060	−0.201	<0.0001	41.06	Ca/Sr, δ^2H, K, Sr, δ^{13}C, Mn, Ca, K/Rb, δ^{15}N, As
M3: Stable isotopes and elemental analysis with δ^{18}O and δ^{34}S	0.649	0.745	0.711	0.045	−0.184	<0.0001	41.88	δ^2H, δ^{18}O, K, δ^{13}C, Sr, Mn, Ca, As, δ^{15}N, Mg
M4: Stable isotopes and elemental analysis with δ^{18}O, δ^{34}S, Rb/Sr, K/Rb, and Ca/Sr	0.681	0.810	0.771	0.069	−0.198	<0.0001	44.18	Ca/Sr, δ^2H, K, Sr, δ^{18}O, δ^{13}C, K/Rb, Mn, Ca, δ^{15}N, As

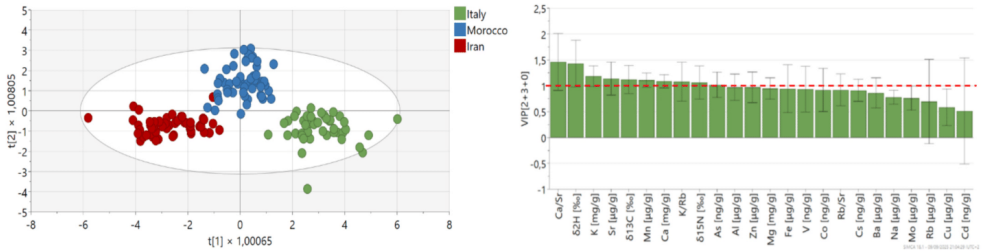


Fig. 3. OPLS-DA models differentiating geographical origins (Italy, Morocco, and Iran) based on isotopic ratios and elemental composition with Rb/Sr, K/Rb, and Ca/Sr excluding δ^{18}O and δ^{34}S values. Score plots (left) illustrate sample clustering by country: Italy (green), Morocco (purple), and Iran (red), with the 95 % confidence interval ellipse. Variable Importance in Projection (VIP) plots (right) highlight the most influential variables (VIP > 1, red dashed line) for group discrimination. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

referred to as alternative sets. The target sets were randomly divided into a training set (70 % of data from the primary dataset) and a test set (30 % of data from the primary dataset) to assess the model's validity. This value corresponds to a 4–5 fold cross-validation and represents a good compromise between having sufficient data to build a good model from the test dataset and the test dataset's error estimate (Gholamy et al., 2018). Also, the Procrustes cross-validation (Kucheryavskiy, Zhilin, Rodionova and Pomerantsev, 2020) was used for model optimization since it is a better alternative to regular cross-validation for short datasets. The exploratory data analysis was performed using the entire dataset with selected variables to explore the data structure and analyze the figure of merit (FoM) results concerning the number of PCs. A classification model was then built using the target set, the alternative set (samples from abroad) and the PCA model with a selected number of PCs. Sensitivity and specificity were used as figures of merit obtained from the target and alternative set.

Isotopic and elemental data, including δ^2H, δ^{13}C, δ^{15}N, and elemental concentrations such as Mg, Ca, Na, Fe, and several trace elements (e.g., Rb, Ba, Sr), were used to build the models for saffron samples originating from Italy, Morocco, and Iran. The selection of variables was optimized for each model. The three models showed excellent specificity (93.6–99.0 %) and sensitivity (94–99 %) across the data tested from Italian, Moroccan and Iranian samples (Table 4).

The model successfully identified whether saffron samples were of Italian origin. Two saffron samples fell outside the acceptance range for authentic Italian saffron and were thus classified as non-Italian (Fig. 4; 1A), which may reflect natural variability within Italian saffron. Further validation using authentic foreign samples from Iran, Morocco, Spain, Slovenia, and the United Kingdom confirmed the model's accuracy. However, one Moroccan sample was misclassified as Italian, suggesting an overlap in isotopic and elemental characteristics (Fig. 4; 1B).

This misclassification may stem from shared environmental factors, such as soil composition, climate, or agricultural practices. When verifying market samples labeled as originating from Spain, Italy, Iran, Greece, and Unknown sources, all were classified as non-Italian, including two samples explicitly labeled as Italian (Fig. 4; 1C) (Table S1). For Moroccan saffron classification, one authentic Moroccan saffron sample fell outside the acceptance range and was classified as non-Moroccan (Fig. 4; 2 A). Additionally, seven foreign saffron samples—two Italian, four Iranian, and one Greek—were misclassified as Moroccan (Fig. 4; 2B). Such finding suggests that saffron from these regions share chemical similarities with Moroccan saffron, making precise classification more challenging. When verifying market samples labeled as originating from Greece, Iran, Spain, Italy, and Unknown sources, all except three commercial Greek samples were correctly classified as non-Moroccan (Fig. 4; 2C). This raises concerns about potential misrepresentation in the saffron trade, where Moroccan saffron could be falsely marketed as Greek or vice versa, misleading consumers and undermining fair trade practices.

The model successfully identified the majority of authentic Iranian

Table 4
Summary of DD-SIMCA models used for distinguishing saffron samples according to the country of origin based on selected variables.

Country	Elements included	No. PC	TP	FN	TN	FP	Specificity (%)	Sensitivity (%)	TEFF (%)
Italy	δ^2H, δ^{13}C, δ^{15}N, Mg, K, Ca, Al, Mn, Fe, Cu, Zn, Rb, Ba, Sr, Mo, V, Co, Cd, As, Cs	4	38	2	117	1	99.0	95.0	97.0
Morocco	δ^2H, δ^{13}C, δ^{15}N, Mg, K, Al, Mn, Fe, Cu, Zn, Ba, Sr, Mo, V, Co, Cd, Cs	4	48	1	102	7	93.6	98.0	95.8
Iran	δ^2H, δ^{13}C, δ^{15}N, Mg, Ca, Na, Al, Mn, Fe, Rb, Ba, Sr, Mo, V, Co, Cd, As	4	46	1	104	6	94.5	97.9	96.0

PC – principal components; TP - a true positive; TN - a true negative; FP - a false positive; FN - a false negative; TEEF – total efficiency.

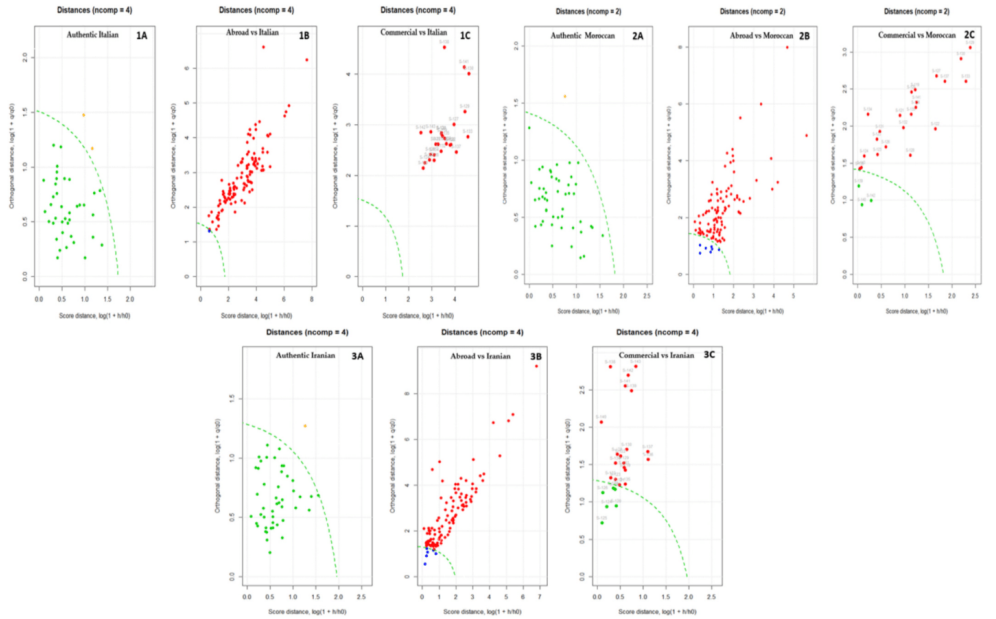


Fig. 4. DD-SIMCA classification model of a) authentic saffron samples (1 A, Italian, $n = 44$; 2 A, Moroccan, $n = 53$; 3 A, Iranian, $n = 52$), b) saffron samples cultivated outside the respective authentic regions (1B, Abroad vs Italian, $n = 118$; 2B, Abroad vs Moroccan, $n = 109$; 3B, Abroad vs Iranian, $n = 110$), c) commercial saffron samples (1C, Italian, $n = 25$; 2C, Moroccan, n = 25; 3C, Iranian, n = 25). The green dashed line represents the 100(1-α) % acceptance area, i.e., the decision boundary determining whether a sample belongs to the target class. Green points in a) indicate correctly identified authentic samples, while orange points represent wrongly rejected authentic samples based on the α value. Red points in b) denote true positive samples from abroad sources, while blue points indicate misclassified authentic samples. In c), red points represent commercial samples not falling within the authentic boundary, and green points represent misclassified commercial samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

saffron samples. However, one authentic Iranian sample was classified as non-Iranian (Fig. 4; 3 A). Additionally, six foreign samples—three Italian, two Moroccan, and one Spanish—were misclassified as Iranian (Fig. 4; 3B). When verifying market samples, all were classified as non-Iranian, except for six commercial Spanish samples and one unknown sample (Fig. 4; 3C). The misclassification of Spanish saffron as Iranian can be attributed to both natural isotopic and elemental similarities— as observed in previous results (Fig. 2), where authentic Iranian and Spanish samples exhibited significant overlap—and/or fraudulent mislabeling. This issue is particularly concerning given reports of large-scale saffron fraud in Spain, where Iranian saffron has been deliberately adulterated and sold as Spanish saffron to command higher prices (Europol, 2021). Interestingly, all six commercial samples labeled as Iranian fall outside the range of authentic samples, suggesting potential mislabeling.

3.3.1. *Quantitative assessment of misclassification*

To assess the extent of potential mislabeling, a total of 75 commercial saffron samples were evaluated using the classification model. The overall misclassification rate was calculated as:

$$\text{Misclassification Rate (\%)} = (\text{Total Misclassified Samples}/\text{Total Samples}) \times 100$$

Table 5
Potential mislabeling of commercial saffron samples by country of origin based on DD-SIMCA classification results.

Country	Total Samples	Misclassified	% Potential Mislabeling
Italy	25	2*	8 %
Morocco	25	3	12 %
Iran	25	6	24 %

* Two commercial samples explicitly labeled as Italian fell outside the authentic Italian range.

Among the analyzed samples, 11 were misclassified, corresponding to an overall misclassification rate of 14.7 % (~15 %). A country-specific breakdown (Table 5) revealed considerable variation, with the highest rate observed in samples labeled as Iranian (24 %). This finding is consistent with earlier reports of widespread saffron fraud, particularly involving the substitution or adulteration of Iranian saffron marketed as Spanish saffron to command higher market value.

These results highlight not only the robustness of the developed models in detecting inconsistencies but also the ongoing challenges in ensuring authenticity across the saffron supply chain. The observed misclassifications may reflect both natural overlaps in isotopic and elemental signatures between regions and deliberate mislabeling practices.

4. Conclusions

Saffron, one of the most valuable spices worldwide, is especially vulnerable to fraud and mislabeling, making authenticity verification essential for consumer trust and fair trade. This study demonstrates that stable isotope and multi-elemental analysis, integrated with advanced statistical modeling, offers a powerful and reliable tool for tracing saffron origin. By analyzing 163 authentic saffron samples-including 75 recently collected from Morocco, Greece, the United Kingdom, Slovenia, Spain, and Iran, together with two previously published datasets that were incorporated due to their strong methodological and analytical comparability-we achieved a robust classification accuracy of 94 %. Key isotopic and elemental markers (δ^2H, δ^{15}N, K, Ca, δ^{13}C, Sr, Mn, Co, and Zn), along with elemental ratios (Rb/Sr, K/Rb, Ca/Sr) and/or δ^{18}O and δ^{34}S, provided strong discriminatory power, enabling precise differentiation particularly for Moroccan saffron. The results further show that either elemental ratios or absolute elemental concentrations can be applied, depending on laboratory instrument availability. While δ^{18}O and δ^{34}S can sometimes be more challenging to obtain due to analytical constraints, their absence can be compensated by robust multi-elemental and other isotopic markers. Moreover, datasets from the literature can be integrated when analytical methods and stable isotope approaches are comparable, with stable isotope data generally offering greater robustness and consistency than elemental composition alone.

Application of DD-SIMCA further confirmed high specificity (95–98 %) and sensitivity (94–99 %) in authenticating saffron origins, while also revealing potential mislabeling in ~15 % of commercial samples. The highest misclassification rate was observed in products labeled as Iranian saffron (24 %), consistent with long-standing concerns about large-scale saffron fraud in this region. These findings reinforce the urgent need for robust authentication frameworks to safeguard traditional producers, enhance transparency in the saffron supply chain, and protect consumers from fraudulent practices.

Future work should focus on expanding global datasets and applying advanced statistical and artificial intelligence (AI) approaches to further improve predictive accuracy, scalability, and adaptability of saffron authenticity verification systems.

CRediT authorship contribution statement

Cathrine Terro: Writing – original draft, Project administration, Data curation, Conceptualization. **Lidija Strojnik:** Writing – review & editing, Visualization, Software, Data curation. **Doris Potočnik:** Methodology, Formal analysis. **Marta Jagodic Hudobivnik:** Validation, Data curation. **Darja Mazej:** Validation, Data curation. **Luana Bontempo:** Writing – review & editing, Resources, Formal analysis. **Mahmoud Eddabdouby:** Resources. **Nour Eddine Amenzou:** Resources. **Benmansour Moncef:** Resources. **Simon Kelly:** Resources, Project administration. **Nives Ogrinc:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2025.103196.

Data availability

Data will be made available on request.

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Corrigendum

Corrigendum to "Stable isotope and multi-elemental analysis combined with statistical modeling for saffron origin authentication" [Food Chem.: X 31 (2025) 103196]

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The authors regret an error in the published article.

The first estimation in the first version of the paper has been wrongly evaluated. After the suggestion of the reviewer the calculation has been corrected:

Answer to the reviewer:

Question 8.

Line 510, 534: The abstract, DD-SIMCA discussion (line 510), and conclusion (line 534) state potential mislabeling "up to 60%". This is a striking figure and requires clear, explicit justification based on the DD-SIMCA results presented.

Response: We agree that the 'up to 60 %' statement was misleading and was incorrectly evaluated. The text has been revised to accurately reflect the actual misclassification rates, as shown in Table 5. Specifically, 14.7 % of commercial samples were misclassified, with the highest rate observed in the Iranian commercial group at 24 %. The 'up to 60 %' statement has been removed, and the correct figure of 14.7 % is now reported.

Table 5
Potential mislabeling of commercial saffron samples by country of origin based on DD-SIMCA classification results.

Country	Total samples	Misclassified	% Potential mislabeling
Italy	25	2*	8 %
Morocco	25	3	12 %
Iran	25	6	24%

* Two commercial samples explicitly labeled as Italian fell outside the authentic Italian range.

Correction in the text:

3.3.1 Quantitative assessment of misclassification

To assess the extent of potential mislabeling, a total of 75 commercial saffron samples were evaluated using the classification model. The overall misclassification rate was calculated as:

$$\text{Misclassification Rate (\%)} = (\text{Total Misclassified Samples}/\text{Total Samples}) \times 100.$$

Among the analyzed samples, 11 were misclassified, corresponding to an overall misclassification rate of 14.7% (~15%). A country-specific breakdown (Table 5) revealed considerable variation, with the highest rate observed in samples labeled as Iranian (24%). This finding is consistent with earlier reports of widespread saffron fraud, particularly involving the substitution or adulteration of Iranian saffron marketed as Spanish saffron to command higher market value.

These results highlight not only the robustness of the developed models in detecting inconsistencies but also the ongoing challenges in ensuring authenticity across the saffron supply chain. The observed misclassifications may reflect both natural overlaps in isotopic and elemental signatures between regions and deliberate mislabelling practices.

The authors would like to apologise for any inconvenience caused.

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3.3 Scientific Report: "Combining Stable Isotope and Elemental Signatures with Machine Learning for Saffron Origin Verification"

In this chapter, I present the study titled "Combining Stable Isotope and Elemental Signatures with Machine Learning for Saffron Origin Verification" by Cathrine Terro, Jan Drole, Tome Eftimov, and Nives Ogrinc. This work was conducted in collaboration with the Faculty of Computer and Information Science, University of Ljubljana, through Jan Drole, and the Computer Systems Department, Jožef Stefan Institute, through Dr. Tome Eftimov.

This study analyzed 115 authenticated saffron samples from Iran (n=51), Italy (n=44), Morocco (n=12), and Spain (n=8), characterized by five stable isotope ratios (δ^{2}H, δ^{13}C, δ^{15}N, δ^{18}O, δ^{34}S) and 42 elemental concentrations. Data preprocessing included median imputation, z-score normalization, and SMOTE (k=3) for class imbalance, with four feature portfolios (isotopes only, elements only, combined) tested across four ensemble algorithms (RF, XGBoost, LightGBM, CatBoost) using stratified 5-fold CV and Bayesian optimization (320 configurations).

LightGBM with isotope and elements achieved optimal performance: 93.07% accuracy (±2.88%), 90.11% balanced accuracy, F1=0.934, with per-class accuracies of 94.1% (Iran), 93.2% (Italy), 91.7% (Morocco), 100% (Spain). SHAP analysis further enhanced the interpretability of the model, offering a transparent view of how each feature contributed to the classification decision. SHAP analysis identified the top elemental discriminators (Ni, Co, K, Mn, Sr, Rb, Ga, Al, Cd, Pb), confirming the superiority of elements over isotopes and enabling a 79% cost reduction through targeted ICP-MS screening while maintaining >90% accuracy.

My contribution to this chapter involved gathering, curating, and organizing the dataset and writing the first draft. This work was presented at the International Mass Spectrometry Conference (IMSC) 2024 in Melbourne, Australia, in the form of a poster.

Combining Stable Isotope and Elemental Signatures with Machine Learning for Saffron Origin Verification

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ABSTRACT

Food fraud remains a global concern, undermining consumer trust and food system integrity. This study integrates stable isotope ratios and multi-element composition data with machine learning to improve saffron authentication and enhance food fraud detection. A total of 115 authenticated saffron samples from four geographical origins (Iran, Italy, Morocco, and Spain) were analyzed across 47 chemical features, including five stable isotope ratios and forty-two elemental concentrations. Four ensemble algorithms—Random Forest, XGBoost, LightGBM, and CatBoost—were evaluated using stratified cross-validation with Bayesian hyperparameter optimization. The combined isotopic and elemental feature set analyzed by LightGBM achieved the best performance (93.07% accuracy, 90.11% balanced accuracy, F1 = 0.934), demonstrating robust classification despite class imbalance. SHAP explainability analysis revealed that elemental markers, particularly nickel and cobalt, were dominant discriminators of geographical origin, while stable isotopes had minimal impact. These findings highlight the superior utility of multi-elemental analysis for cost-effective and reliable food authentication. The proposed model provides a scalable, interpretable framework for detecting geographical mislabeling and improving transparency in food supply chains.

Introduction

Food fraud is a persistent global concern, with experts estimating that it affects approximately 1% of the global food industry, with a global economic impact estimated at USD 30–65 billion annually [1,2,3,4]. This issue affects not only consumers and their trust but also honest producers, processors, retailers, and regulatory bodies [5]. Food fraud continues to pose threats to the dynamics within the agri-food systems, impairing the food safety and trust in these systems [6]. Several recent scandals and high-profile food crime cases such as the olive oil fraud [7] and the fake saffron scandal [8] have highlighted this problem and piqued public interest and attention. These incidents emphasize the broader issue of food fraud which encompasses misleading practices and intentional alterations of food products and ingredients, often for economic gain. While the primary motivation is financial, its effects pose significant threats to public health and well-being. The act of committing food fraud consists of tampering, addition, substitution, or mislabelling and misrepresenting of food, the ingredients, or even the packaging where false or deceptive claims about food products are made [9]. There are seven types of fraud that have been identified as adulteration, tampering, overrun, theft, diversion, simulation, and counterfeiting[10]. Addressing food fraud starts with confirming the authenticity of food products. Technological developments have increasingly enhanced the capacity to detect food fraud, aid in outbreak investigations, improve the predictability for identifying potential risks and reinforce the traceability in the food supply chain. The food sector is experiencing fast evolutions in these areas [6].

Proof of provenance has become an important topic in food safety, food quality and consumer protection in accordance with national legislation and international standards and guidelines. Enhancing food traceability simplifies the tracking of adulterators, provides faster responses to fraud incidences, protects product origins, and reinforces trust in food products and processes across the supply chain [11]. Traceability systems are crucial in combating fraud with stable isotopes and elemental analysis emerging as some of the most effective methods currently being used. Stable isotope ratios of light elements allow the relationships between isotope ratios and the fractionation processes associated with local climate data, as well as plant and animal physiology, geology, and pedology, to be analysed. This approach transfers isotopic signals of light elements (H, C, N, O, and S) from different natural sources (water, soil, and atmosphere) to plant and animal tissues. Measurements of the stable isotope ratios of these elements are expressed in the -notation in ‰, calculated as $iE = (R(iE/jE)\ sample / R(iE/jE)\ standard) - 1$, where i and j represent the highest and lowest atomic mass numbers of the element E (C, N, O, S), and R is the ratio between the heavier and lighter isotope ($^2H/^1H$, $^{13}C/^{12}C$, $^1N/^1N$, $^1O/^1O$, $^3S/^{32}S$) in the sample or standard; ^{13}C values are reported relative to the V-PDB (Vienna-Pee Dee Belemnite) standard, ^1N values relative to AIR, ^3S values relative to V-CDT (Vienna-Canyon

Diablo Troilite), and ^{2}H and ^{1}O values relative to VSMOW (Vienna-Standard Mean Ocean Water).

For instance, the ^{2}H and ^{18}O values of water can provide critical information about the origin of water (e.g., local precipitation, surface water, and groundwater) that depend on latitude, altitude, distance from the sea, amount of precipitation and the degree of evapotranspiration [12]. In plants, the ^{13}C value is mainly regulated by the plants' specific CO_2-fixation pathway (e.g., C3 or C4 plants). However, other factors include atmospheric CO_2 concentration, plant variety, physiology, the cell's nutritional status, growth rate, water-use efficiency, and cultivation practices. While ^{15}N values have been widely used to distinguish between organic and conventional products, it has also proven valuable in geographical origin studies [13]. The distinctive ^{15}N value of plants depends on the soil's ^{15}N value and is influenced by the local climate, general soil conditions, long-term soil treatment, and land use [14]. The ^{34}S values reflect geology, volcanism, the influence of sea spray (i.e., distance from the sea), and specific anthropogenic effects, e.g., such as the source of atmospheric SO_2 [15], can also act as local markers [13]. The verification of regional origin can be even more effective when stable isotopes are combined with elemental composition since a plant's elemental profile is related to the soil composition of the location where the plant grows; consequently, bioavailable nutrients can provide direct information about an agricultural products' geographical origin [16].

The traceability system based on isotopic and elemental composition relies on comprehensive databases of authentic reference samples. These databases must capture the full range of values for authentic food products from specific locations, accounting for variations in farming practices, husbandry, and environmental conditions. However, creating and maintaining such databases is challenging due to the extensive variation that must be included. To evaluate authenticity, values found in commercial samples are compared with the limits derived from the dataset's contents and assessed for best fit using a suitable statistical model. The European Commission has maintained an isotopic database for wine for over 20 years, which helps determine geographic origin [17]. Nevertheless, other databases are not as well developed. While some European databases are freely available, many are not shared due to intellectual property issues and differences in sample pre-treatment methods. At the Jožef Stefan Institute, the IsoFoodTrack database [18](www.isofoodtrack.ijs.si) was developed for different commodities of interest, such as spices, milk and dairy products, oils, and meat.

Statistical models play a crucial role in analysing, understanding and interpreting the complex data from traceability systems. Various statistical evaluations and models are utilized to handle complicated datasets and determine the authenticity and origin of foods. Conventional methods used include exploratory analysis such as Principal Component Analysis (PCA) and cluster analysis, as well as classification methods like Discriminant Analysis (DA), K-Nearest Neighbors (k-NN) and Partial Least Squares-Discriminant Analysis (PLS-DA). For confirming a sample's regional origin, class modeling, rather than discriminant analysis, is often recommended due to possible biases in one-class classification problems. Soft independent modeling of class analogy (SIMCA) is a common method in chemometrics for such tasks [19,20,21].

Although classical statistical approaches have historically been used in food traceability research, machine learning (ML) methods are now increasingly employed and gaining popularity. In most cases, these studies use isotopic measurements of food samples to predict or classify their geographic origin, employing classification models such as Random Forest, XGBoost, or Neural Networks and comparing their performance metrics. Despite an abundance of published literature and growing pressure from reviewers to include these analyses, many studies simply apply ML models without adhering to a rigorous experimental design or proper benchmarking, highlighting the need for standardized methods in ML-based food traceability research.

To address the aforementioned challenges and provide deeper insights into food traceability, we propose a novel ML-based framework consisting of the following steps: (1) selecting representative training data, (2) performing feature selection to identify measurements that improve model performance without overfitting, (3) training the model, (4) evaluating the model using diverse train–test splits to ensure robust results, and (5) providing sample-specific explanations through feature importance.

In our study, we combined stable isotopic and multi-elemental composition data of saffron samples, along with key metadata (parameters) to enhance the verification models for food authentication and provide a viable tool in ensuring food safety and integrity. There have been studies that use stable isotopes and multi-elemental analysis for saffron authentication, however integration of AI is novel. Most current research focuses on individual methods for differentiating saffron origins. Our paper distinguishes itself from other studies by providing a multi-faceted and interdisciplinary authentication approach. By combining sophisticated analytical techniques with AI, we offer an accurate, scalable approach for ensuring product authenticity and advancing food fraud detection.

Methods

Dataset Description

The database used in this evaluation compiles saffron data from the literature, trusted collaborators, and our own samples, and is maintained within the IsoFoodTrack database [18]. Extensive metadata are available for each sample, including dates or years of production (2006–2023), type of production (farm or commercial), country of origin, production region or province, specific location name, geographic coordinates (latitude °, longitude °), altitude (m), annual mean temperature (°C), annual mean precipitation (mm), and distance from the coast (km) corresponding to the location where the saffron was grown and harvested. Details of the analytical procedures and quality control measures are provided in previous publications [22,23,24,25].

From this larger resource, a subset of authenticated saffron samples was selected for the present study. The dataset consists of 115 saffron samples originating from four countries: Iran (n = 51), Italy (n = 44), Morocco (n = 12), and Spain (n = 8). Each sample was characterized using a comprehensive chemical profiling approach combining stable isotope ratio analysis and elemental composition analysis. Specifically, the dataset includes stable isotopic data for five isotopes ($\delta^{2}H$, $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, $\delta^{34}S$; unit ‰) and elemental concentration data for 42 elements (Na, Mg, Al, K, Ca, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Rb, Cd, Ba, Pb, As, Cs, Sr, Li, Be, B, Sc, Ti, Ga, Y, Zr, Nb, Mo, Ag, La, Ce, Pr, Nd, Sm, Eu, Dy, Ho, Er, Hf, Re; units mg/g and/or ng/g).

The dataset exhibited a pronounced class imbalance across geographical origins. Samples from Iran and Italy accounted for 82.6% of the total dataset, whereas Morocco and Spain were underrepresented, with Spain constituting only 7.0% of the samples. This imbalance was explicitly considered during subsequent data analysis and model development.

Geographical origin classification

The classification workflow was organized into five key phases: data preprocessing, feature portfolio design, model training, cross-validation and class imbalance handling, and hyperparameter optimization.

Data Preprocessing. In this step the dataset was cleaned up, since it was compiled from multiple sources, it was essential to standardize the categorical data. This involved removing extraneous whitespaces and ensuring consistent letter casing across all entries. Missing values were imputed using median imputation for each feature independently. All features were subsequently standardized using z-score normalization (zero mean, unit variance) to ensure equal contribution across different measurement scales. To address class imbalance, Synthetic Minority Over-sampling Technique (SMOTE) with k=3 nearest neighbors was applied exclusively to training sets within each cross-validation fold, preventing data leakage and maintaining test set integrity.

Feature Portfolio Design. To evaluate the individual and combined contributions of isotopic and elemental information to geographical classification, four distinct feature portfolios were defined and systematically assessed. The first portfolio comprised only stable isotope ratios, including the five isotopes. The second portfolio included exclusively elemental composition data, consisting of concentrations for 42 elements. The third portfolio combined both isotopic and elemental features, resulting in a total of 47 variables. Finally, the complete feature portfolio included all available chemical descriptors, encompassing both isotopic and elemental measurements, for a total of 47 features.

Machine Learning Algorithms. Four ensemble learning algorithms were evaluated to assess their classification performance. Random Forest (RF) was employed as a bagging-based ensemble method using decision tree base learners. In addition, three gradient boosting approaches were considered: eXtreme Gradient Boosting (XGBoost), which implements a tree-based gradient boosting framework; Light Gradient Boosting Machine (LightGBM), designed for high computational efficiency through histogram-based learning; and Categorical Boosting (CatBoost), which incorporates ordered boosting and is optimized to handle categorical features while reducing prediction bias.

Cross-Validation Strategy. A stratified five-fold cross-validation strategy was employed to ensure that the class distribution of geographical origins was preserved across both training and test sets in each fold. For every iteration, the dataset was partitioned into a training set comprising 92 samples and an independent test set of 23 samples. To mitigate the effects of class imbalance, the Synthetic Minority Over-sampling Technique (SMOTE) was applied exclusively to the training data within each fold, while the corresponding test set was left unaltered to prevent data leakage and ensure unbiased model evaluation. This cross-validation procedure resulted in a total of 115 predictions across all folds, with each sample serving as a test instance exactly once.

Hyperparameter Optimization. Bayesian hyperparameter optimization was implemented using the Optuna framework to systematically identify optimal parameter configurations for each machine learning algorithm and feature portfolio combination. For each model, algorithm- specific search spaces were defined to reflect the structural and computational characteristics of tree-based ensemble methods. Balanced accuracy was selected as the optimization objective in order to

appropriately account for the pronounced class imbalance present in the dataset.

For every algorithm–portfolio combination, 20 optimization trials were conducted, with model performance evaluated using stratified five-fold cross-validation within each trial to ensure robust and reliable performance estimates. This optimization strategy resulted in a total of 320 evaluated model configurations (4 algorithms × 4 feature portfolios × 20 trials). The key hyperparameters explored during optimization included the number of estimators (50–500), maximum tree depth (3–15), learning rate (0.01–0.3), minimum number of samples per leaf (1–10), and subsample ratio (0.5–1.0), all of which are known to strongly influence model complexity, generalization performance, and computational efficiency.

Results and Discussion

Overall Classification Performance

Table 1 presents the comprehensive performance comparison across all algorithm-portfolio combinations. The results demonstrate marked performance variation depending on feature composition and algorithm selection.

Feature Portfolio	Algorithm	Accuracy (%)	Bal. Acc. (%)	F1 Score	Std Dev
Isotopes Only	Random Forest	13.02	33.55	0.098	6.89
	XGBoost	21.52	29.76	0.134	21.69
	LightGBM	**44.35**	25.00	0.273	0.66
	CatBoost	13.02	**33.55**	0.098	6.89
Elements Only	Random Forest	92.17	85.05	0.919	2.63
	XGBoost	89.61	82.55	0.888	6.81
	LightGBM	**93.97**	**88.32**	**0.941**	5.88
	CatBoost	93.07	85.54	0.929	3.90
Isotopes + Elements	Random Forest	93.05	85.54	0.927	4.00
	XGBoost	89.61	82.55	0.888	6.81
	LightGBM	**93.07**	**90.11**	**0.934**	**2.88**
	CatBoost	93.95	89.71	0.939	5.37

Table 1. Classification performance across feature portfolios and algorithms. Best performance per portfolio highlighted in bold.

Optimal Model Selection: LightGBM with Isotopes and Elements

Based on balanced accuracy, stability (low standard deviation), and practical interpretability, LightGBM with Isotopes and Elements features was selected as the optimal model for saffron origin classification. This model achieved 93.07% accuracy ± 2.88%, 90.11% balanced accuracy ± 6.05%, and F1 score of 0.934. The low standard deviation across folds indicates robust and consistent performance, crucial for real-world application in food authentication.

	Pred. Iran	Pred. Italy	Pred. Morocco	Pred. Spain
Actual Iran	**48**	3	0	0
Actual Italy	2	**41**	1	0
Actual Morocco	0	1	**11**	0
Actual Spain	0	0	0	**8**

Table 2. Confusion Matrix (Aggregated across 5 folds, 115 total predictions). Per-class performance: Iran 94.1% (48/51), Italy 93.2% (41/44), Morocco 91.7% (11/12), Spain 100% (8/8).

SHAP Explainability Analysis

To elucidate the decision-making process of the optimal model, SHAP (SHapley Additive exPlanations) analysis was performed. SHAP values quantify each feature's contribution to individual predictions, providing both global feature importance rankings

and local explanations for specific classifications.

Global Feature Importance. Figure 1 presents the aggregated SHAP feature importance across all 115 predictions and four classes, ranked by mean absolute SHAP value. The analysis reveals a clear hierarchy of discriminatory features for saffron origin classification.

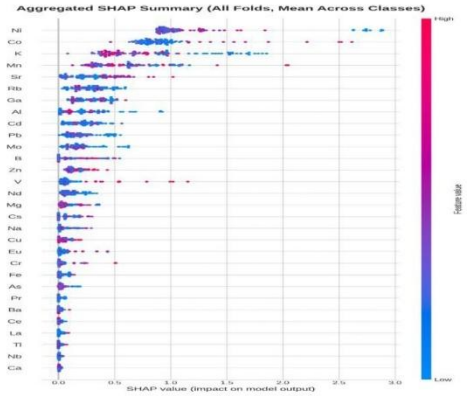


Figure 1. Global SHAP feature importance for saffron origin classification. Beeswarm plot showing top 30 features ranked by mean absolute SHAP value. Each point represents a sample; color indicates feature value (red=high, blue=low); x-position indicates SHAP contribution.

Feature importance analysis revealed that the ten most influential variables contributing to the classification model were exclusively elemental concentrations. Nickel (Ni) emerged as the dominant discriminatory feature, exhibiting the highest importance value (1.226), followed by cobalt (Co) with a substantial contribution (0.950). Potassium (K), an essential macronutrient known to vary with soil composition and agricultural practices, ranked third (0.839). Other influential elements included manganese (Mn; 0.604), strontium (Sr; 0.313), rubidium (Rb; 0.300), gallium (Ga; 0.294), aluminum (Al; 0.234), cadmium (Cd; 0.210), and lead (Pb; 0.209). Many of these elements are closely associated with regional soil geochemistry and environmental conditions, supporting their relevance for geographical discrimination.

Notably, all five stable isotope ratios exhibited near-zero SHAP values (0.001), indicating a negligible contribution to model predictions within this dataset. This finding suggests that, in contrast to elemental composition, isotopic signatures provided limited discriminatory power for the classification task. The prominence of nickel and cobalt in particular likely reflects distinct geological substrates and region-specific agricultural practices across the saffron-growing areas considered in this study.

Per-Class SHAP Patterns. Figure 2 presents class-specific SHAP beeswarm plots, revealing how features differentially contribute to classification of each geographical origin.

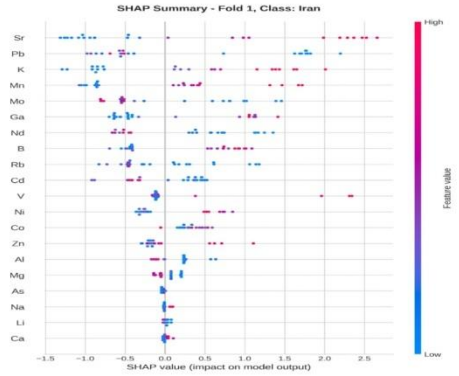


Figure 2A. SHAP importance for Iran classification. High Ni, Co, and Mn favor Iranian origin.

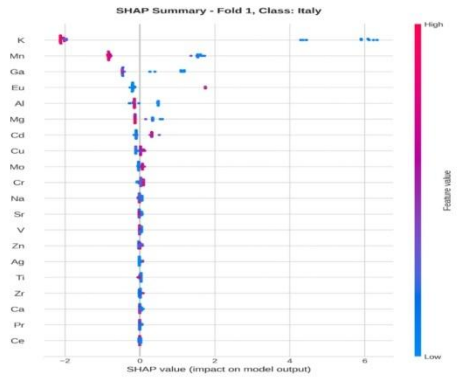


Figure 2B. SHAP importance for Italy classification. Elevated Sr, Rb, Ga favor Italian origin; high Ni/Co oppose.

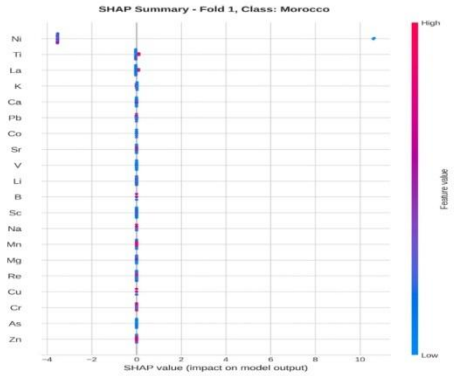


Figure 2C. SHAP importance for Morocco classification. Moderate values across features; intermediate compositional space.

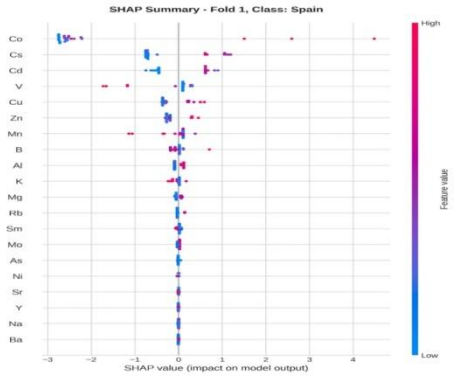


Figure 2D. SHAP importance for Spain classification. Distinctive Pb, Sr, K profiles; high Ni opposes Spanish classification.

Local Explanations: Individual Prediction Decomposition

Figure 3 presents SHAP waterfall plots for representative correctly classified samples from each geographical origin, illustrating how features combine to produce individual predictions. Each plot shows the contribution of the top 20 features, with red bars pushing toward the predicted class and blue bars pushing away.

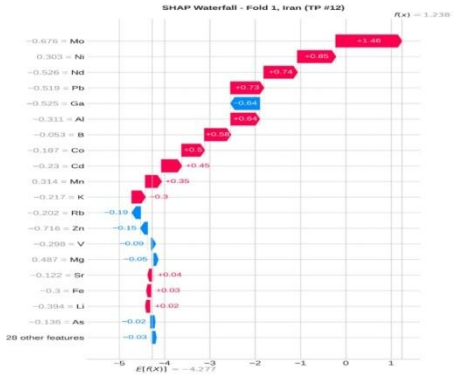


Figure 3A. SHAP waterfall for the Iranian samples. High Ni (+0.89), Co (+0.67), Mn (+0.41) drive classification.

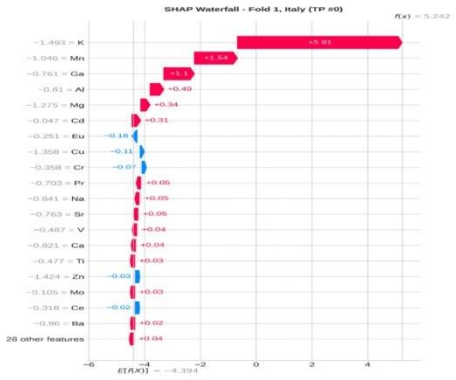


Figure 3B. SHAP waterfall for Italian samples. Sr, Rb, Ga push toward Italy; low Ni/Co reinforce.

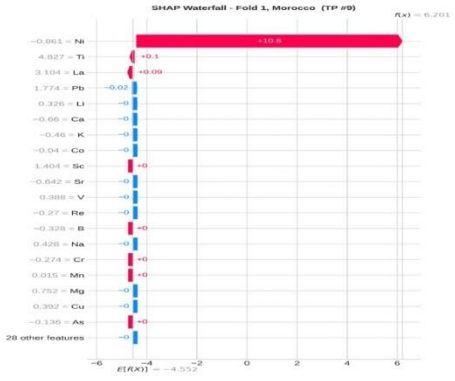


Figure 3C. SHAP waterfall for Moroccan samples. Balanced contributions from multiple features.

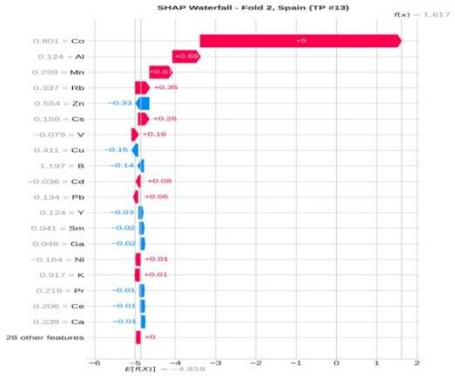


Figure 3D. SHAP waterfall for the Spanish samples. Distinctive Pb-Sr signature enables perfect discrimination.

Conclusions

This study demonstrates that machine learning classification of saffron geographical origin achieves 93.07% accuracy using combined stable-isotope and elemental analysis, with LightGBM as the optimal algorithm. SHAP explainability analysis revealed that elemental concentrations, particularly nickel and cobalt, provide the primary discriminatory information, while stable isotopes contribute negligibly to classification performance.

The distinct geochemical fingerprints identified for each origin: Iran's transition metal enrichment, Italy's strontium-rubidium signature, Morocco's intermediate profile, and Spain's distinctive lead-strontium pattern, provide objective, quantifiable criteria for regulatory enforcement. The model's high balanced accuracy (90.11%) despite severe class imbalance demonstrates robustness suitable for real-world applications.

Key recommendations include: (1) prioritization of multi-element ICP-MS analysis over costly isotope ratio mass spectrometry for routine authentication, (2) focus on the top 10 discriminatory features for cost-effective screening (Ni, Co, K, Mn, Sr, Rb, Ga, Al, Cd, Pb), potentially reducing analytical costs by 79% while maintaining >90% accuracy, and (3) adoption of SHAP-based explainability to provide auditable evidence for authentication decisions in regulatory contexts.

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3.4 Scientific Paper: "Assessing the Authenticity and Quality of Paprika (Capsicum annuum) and Cinnamon (Cinnamomum spp.) in the Slovenian Market: A Multi-Analytical and Chemometric Approach"

In this chapter, I present the paper titled "Assessing the Authenticity and Quality of Paprika (*Capsicum annuum*) and Cinnamon (*Cinnamomum spp.*) in the Slovenian Market: A Multi-Analytical and Chemometric Approach" by Sabina Primožič, Cathrine Terro, Lidija Strojnik, Nataša Šegatin, Nataša Poklar Ulrih, and Nives Ogrinc. The paper was published in Foods in 2025 as part of the Special Issue "*Chemometrics in Food Chemistry and Analysis: Novel Detection Methods to Assess Food Quality and Safety*". This paper resulted from collaborative work between Sabina Primožič and me, with both of us contributing as co-authors.

This study represents the first application of stable isotope-based authentication to cinnamon. By applying this framework to Slovenian market samples, we aimed to establish a reliable, multi-dimensional model to support future spice authentication and traceability initiatives. The study investigated 45 paprika and 46 cinnamon samples from the Slovenian market to assess authenticity and quality, including geographic origin, production practices, and potential adulteration. A multi-analytical approach was applied, integrating stable isotope ratio analysis ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$), multi-elemental profiling, FTIR spectroscopy, antioxidant compound analysis, and chemometric classification tools.

Distinct isotopic and elemental markers (e.g., $\delta^{13}C$, $\delta^{34}S$, Rb, Cs, V, Fe, Al) enabled classification of paprika samples by geographic origin with preliminary classification accuracies of 90% (Hungary, Serbia, Spain). Cinnamon samples from Sri Lanka, Madagascar, and Indonesia achieved 95% discrimination accuracy using $\delta^{34}S$ and Ba as key markers, supported by additional elements such as As, Rb, Na, $\delta^{13}C$, S, Mg, Fe, V, Al, and Cu. FTIR analysis distinguished Ceylon from cassia cinnamon and revealed possible adulteration with undeclared additives (e.g., oleoresin removal or azo dyes).

Paprika samples of organic origin showed higher $\delta^{15}N$, $\delta^{34}S$, and Zn values, whereas conventional samples contained higher levels of Na, Al, V, and Cr. Antioxidant profiling supported quality differentiation but indicated variability in phenolic and non-phenolic contributors, especially in cassia cinnamon. Overall, the integration of analytical techniques with chemometrics demonstrated strong potential for authenticating high-value spices, though larger datasets are needed to improve robustness and generalizability.

In this study, I was responsible for the conceptualization of the research, data curation, and investigation. I also contributed to writing the original draft. This work was presented

as a poster at the 8th IMEKOFOODS – Food Safety and Traceability for a Sustainable Future, held from 22–24 September 2025 at the Grand Hotel Union in Ljubljana, Slovenia, where it received the **Best Poster Award**.

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Article

Assessing the Authenticity and Quality of Paprika (*Capsicum annuum*) and Cinnamon (*Cinnamomum* spp.) in the Slovenian Market: A Multi-Analytical and Chemometric Approach

Sabina Primožič [1,†], Cathrine Terro [2,3,†], Lidija Strojnik [2], Nataša Šegatin [1], Nataša Poklar Ulrih [1] and Nives Ogrinc [2,3,*]

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Abstract

The authentication of high-value spices such as paprika and cinnamon is critical due to increasing food fraud. This study explored the potential of a multi-analytical approach, combined with chemometric tools, to differentiate 45 paprika and 46 cinnamon samples from the Slovenian market based on their geographic origin, production methods, and possible adulteration. The applied techniques included stable isotope ratio analysis (δ^{13}C, δ^{15}N, δ^{34}S), multi-elemental profiling, FTIR, and antioxidant compound analysis. Distinct isotopic and elemental markers (e.g., δ^{13}C, δ^{34}S, Rb, Cs, V, Fe, Al) contributed to classification by geographic origin, with preliminary classification accuracies of 90% for paprika (Hungary, Serbia, Spain) and 89% for cinnamon (Sri Lanka, Madagascar, Indonesia). Organic paprika samples showed higher values of δ^{15}N, δ^{34}S, and Zn, whereas conventional ones had more Na, Al, V, and Cr. For cinnamon, a 95% discrimination accuracy was achieved between production practice using δ^{34}S and Ba, as well as As, Rb, Na, δ^{13}C, S, Mg, Fe, V, Al, and Cu. FTIR differentiated Ceylon from cassia cinnamon and suggested possible paprika adulteration, as indicated by spectral features consistent with oleoresin removal or azo dye addition, although further verification is required. Antioxidant profiling supported quality assessment, although the high antioxidant activity in cassia cinnamon may reflect non-phenolic contributors. Overall, the results demonstrate the promising potential of the applied analytical techniques to support spice authentication. However, further studies on larger, more balanced datasets are essential to validate and generalize these findings.

Keywords: paprika; cinnamon; stable isotopes; elemental composition; authentication; traceability; geographical origin; FTIR spectroscopy; phenolics; antioxidant activity

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1. Introduction

Ensuring the authenticity of spices is an urgent challenge in global food safety and trade, as increasing rates of adulteration threaten consumer trust, public health, and the integrity of international markets. In the European Union, routine testing has revealed significant fraud rates: 6% for paprika, 11% for turmeric, 14% for cumin, and 48% for

oregano [1]. The complexity of global supply chains and the growing demand for organic and natural products further complicates efforts to guarantee authenticity and traceability.

Culinary spices such as paprika (*Capsicum annuum*) and cinnamon (*Cinnamomum* spp.) are indispensable to global cuisines, being valued for their distinctive flavors, vibrant colors, and bioactive compounds, which provide notable health benefits. These functional properties make them highly sought after in the food, pharmaceutical, and cosmetics industries, where they are utilized for their antioxidant, antimicrobial, and anti-inflammatory effects [2–4]. Paprika, produced from dried and ground bell peppers, is a staple ingredient in many culinary traditions, particularly in Europe. Although native to Central and South America, paprika is now primarily cultivated in Hungary, Spain, Turkey, Croatia, Serbia, and North Macedonia to meet international demand for both sweet and hot varieties. It grows best in warm, humid climates, which help develop its distinctive color and aroma, but in cooler areas, it must be planted each year because it cannot survive frost [5,6].

Similarly, cinnamon is derived from the bark of evergreen trees of the *Lauraceae* family, which flourish in tropical and subtropical climates where soil and environmental conditions significantly affect its chemical composition and quality [7]. The global cinnamon market is dominated by China, Indonesia, Vietnam, and Sri Lanka, with the latter recognized as the primary producer of the highly valued Ceylon cinnamon (*Cinnamomum verum*), renowned for its delicate flavor and lighter bark. This premium variety is distinguished from the more widely available and lower-cost cassia types (*C. cassia*, *C. aromaticum*), contributing significantly to global trade. The health benefits of cinnamon are attributed to its bioactive constituents, including cinnamaldehyde, eugenol, and cinnamic acid [7].

Given their high economic value, spices like paprika and cinnamon are particularly vulnerable to food fraud, including adulteration with fillers or dyes and mislabeling of origin or species. Such practices pose health risks and undermine consumer trust [8]. Paprika is often subjected to fraudulent practices, such as dilution with inferior materials (e.g., white pepper, curcuma, brick powder), misrepresentation of its geographic origin, and the illegal addition of synthetic dyes like Sudan I and IV to enhance its color [9,10]. The natural degradation of paprika's color during storage, especially in powdered form, further complicates fraud detection, as increased surface area accelerates oxidation and color loss. Similarly, cinnamon is often adulterated by substituting true Ceylon cinnamon with less expensive cassia bark. Cassia naturally contains significantly higher levels of coumarin, which can exceed safety limits and pose health risks with regular consumption [11,12]. Other common adulteration methods include mixing in fillers such as coffee husks and adding synthetic flavor compounds like cinnamaldehyde to mimic authentic cinnamon flavor [13,14]. More alarmingly, recent investigations by the U.S. Food and Drug Administration have uncovered intentional adulteration of ground cinnamon with lead chromate, a toxic colorant added to enhance appearance, leading to multiple public health alerts and product recalls [15–17].

Various analytical techniques have been established to help detect spice adulteration and verify authenticity. Spectroscopic methods, such as Fourier-transform infrared spectroscopy (FTIR), near-infrared (NIR), and X-ray fluorescence (XRF), enable rapid screening for adulterants by generating characteristic chemical profiles of samples. Chromatographic techniques (GC-MS, LC-MS, HPLC) and DNA barcoding further support the identification and quantification of specific adulterants and plant species, especially when combined with chemometric analysis and microscopy for improved detection accuracy [18–23]. In addition to these methods, antioxidant profiling has become increasingly relevant for assessing the quality of spices, as it provides insights into the bioactive compounds present, which can be linked to their geographical origin and production methods, thereby supporting efforts to verify authenticity and traceability [24,25]. However, while effective for detecting

adulterants, these approaches often lack the robustness to distinguish the geographical origin and agricultural production method independently.

Consequently, these factors have been reliably evaluated using profiling or fingerprinting strategies, such as stable isotope and elemental analysis, which, when combined with chemometrics, have proven effective for ensuring accurate traceability and verification. The elemental profile of a spice reflects the soil in which it is cultivated. Additionally, stable isotope ratios of light elements ($^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{34}S/^{32}S$) offer information on plant type or diet (carbon and nitrogen isotopes), geographic origin (hydrogen and oxygen isotopes), and production methods (e.g., organic vs. conventional), serving as robust markers for tracing these factors [26,27]. Previous studies have demonstrated the utility of stable isotope ratios, such as $\delta^{13}C$ and $\delta^{15}N$, for distinguishing geographical origin and cultivation practices, and $\delta^{18}O$ and $\delta^{2}H$ for tracing provenance and water sources in bell pepper (*Capsicum annuum*) [28,29].

Radiogenic isotopes, such as strontium (Sr), also provide valuable information for tracing the geochemical provenance of food products like spices. For example, Brunner et al. [30] demonstrated that combining multi-elemental analysis (including Rb, Sr, Y, Zr, Mo, Cd, Ba, Pb, Th, U, Mg, Ca, Sc, Ti, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, and rare earth elements) with radiogenic strontium isotope ratios ($^{87}Sr/^{86}Sr$) creates a unique fingerprint for authentic Szegedi paprika, effectively determining its geographical origin. The study by Brunner et al. remains the primary and most frequently cited example of integrating multi-elemental profiling and stable isotope ratios for the authentication and determination of paprika's origins. In contrast, most studies on paprika have focused on either multi-elemental or stable isotope analysis in isolation, without combining both approaches within a single investigation.

FTIR further enhances food authentication by providing rapid, non-destructive analysis. By comparing the unique spectral fingerprints of paprika or cinnamon samples with the reference spectra of authentic substances, adulterants such as spent paprika or coffee husks can be detected. FTIR allows for the simultaneous analysis of multiple sample components, offering a comprehensive overview of chemical composition [31]. For instance, Lixourgioti et al. [32] demonstrated the effectiveness of FTIR combined with chemometric classification for authenticating cinnamon and detecting adulteration in the cinnamon supply chain.

Building on the strengths of individual analytical techniques, this study introduces an integrated authentication strategy that combines stable isotope analysis, multi-elemental profiling, FTIR fingerprinting, and complementary antioxidant profiling. This comprehensive approach enables the robust detection of adulteration and precise verification of geographical origin and production methods for paprika and cinnamon. Notably, this is the first application of stable isotope-based authentication to cinnamon. By applying this framework to Slovenian market samples, we aim to establish a reliable, multidimensional model to support future spice authentication and traceability initiatives.

2. Materials and Methods

2.1. Sample Collection

Ninety-one spice samples, consisting of forty-five paprika and forty-six cinnamon samples, were purchased from Slovenian markets and online stores. The selection was diverse regarding type, texture, geographical origin, and agricultural methods, as detailed in the Supplementary Material (Tables S1 and S2).

The paprika samples included sweet, hot, and smoked varieties, with some lacking specific variety identification. Among the 45 samples, 17 were sweet, 12 were hot, 11 were smoked (3 were sweet and 3 were hot), and 5 were of unidentified varieties. The geographi-

cal distribution of the samples was as follows: Hungary (n = 5), Serbia (n = 4), Spain (n = 22), Peru (n = 2), India (n = 1), and China (n = 2). Five samples had no origin information, while three were blends from multiple origins or labeled as EU agriculture. Five samples were labeled as organic.

Cinnamon samples varied in geographical origin, agricultural method, price, packaging weight, and type of packaging. Nineteen were labeled as organic. The origin distribution included Sri Lanka (13), Madagascar (5), Indonesia (8), Vietnam (2), China (1), and India (1). Seven samples had no specified geographical origin, and nine were labeled non-EU agricultural products or mixtures of different origins. Samples included 38 ground cinnamon samples and 8 cinnamon sticks (whole bark). Twenty-three were identified as Ceylon cinnamon and five as cassia.

2.2. Preparation of Extracts

Known weights (5 g) of paprika powder and ground cinnamon sticks were wrapped in cling film and frozen at −80 °C for 1 h. The frozen samples were then lyophilized. For extraction, 3.00 ± 0.01 g of the lyophilized sample was mixed with 15 mL of 70% ethanol using a vortex. The mixture was placed on a shaker for 2 h, with vortex mixing every 30 min. It was then sonicated in an ultrasonic bath for 30 min, with intermittent vortex mixing, followed by an additional 3 h of shaking with vortex mixing every 20 min. Once extracted, the mixture was filtered (Sartorius, Göttingen, Germany) into a 15 mL centrifuge tube covered in aluminum foil. The extracts were centrifuged at 4000 rpm for 10 min, and the supernatant was stored at 4 °C. Before analysis, the extracts were brought to room temperature (25 °C) and centrifuged for 5 min at 4000 rpm.

2.3. DPPH Radical Scavenging Activity Analysis

Antioxidant activity was determined using the DPPH radical scavenging method [33]. In brief, 0.1 mL of extract was diluted with 70% ethanol (15 mL) and added to 2.9 mL of a DPPH (diluted 50-fold for the assay) in ethanol. The DPPH solution was prepared on an ongoing basis, with a freshly prepared working solution to ensure the stability and reliability of the radical concentration, as the DPPH radical concentration decreases over time, even when stored protected from light and in a refrigerator. The mixture was vortexed and incubated in the dark for 30 min at room temperature (25 °C). A control sample (0.1 mL of 70% ethanol + 2.9 mL DPPH solution) was prepared similarly. After incubation, absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Hewlett-Packard, Palo Alto, CA, USA). Color interference was assessed by performing background absorbance (517 nm) measurements of the dark-colored samples. Dark-colored spice matrices can introduce significant matrix interferences in spectrophotometric assays like the DPPH radical scavenging method. These interferences arise from the intrinsic coloration and polyphenolic content of the sample itself, which may absorb at or near the same wavelength used to measure DPPH activity. As a result, the absorbance attributable to DPPH reduction can be confounded by background absorbance from the extract, leading to over- or underestimation of antioxidant activity. To mitigate this, we included sample blanks to correct for background absorbance and used diluted samples to minimize color intensity without exceeding assay linearity. Antioxidant activity was expressed as trolox equivalents (mg trolox per gram of dry sample).

2.4. Total Phenolic Content Analysis

The total phenolic content was determined using the Folin–Ciocalteu method [34] (Gutfinger, 1981). A 0.2 mL aliquot of the extract, diluted in 70% ethanol (15 mL), was mixed with 0.125 mL of Folin–Ciocalteu reagent and left to stand for 5 min. Then, 0.125 mL of 20% sodium carbonate solution was added, and the volume was adjusted to 1 mL with

Milli-Q water. The mixture was vortexed, incubated in the dark at room temperature (25 °C) for 40 min, and centrifuged at 13,000 rpm for 10 min. Absorbance was measured at 765 nm using a UV-Vis spectrophotometer (Hewlett-Packard, Palo Alto, CA, USA). Results were expressed as milligrams of gallic acid equivalents per gram of dry lyophilized sample (mg GA/g LV).

2.5. Fourier-Transform Infrared Spectroscopy (FTIR)

The IR spectra were obtained using Fourier transform infrared spectroscopy (FTIR) with an ATR-FTIR Frontier spectrometer (Perkin Elmer, Shelton, CT, USA). No prior sample preparation was required except for grinding non-powdered paprika and cinnamon samples. All samples were stored in a dry, dark place until analysis. Samples were pressed ensuring the pressure of 60 kp for samples of paprika and 70 kp for cinnamon on the ATR measurement cell to ensure optimal contact with the diamond crystal. Spectra were recorded at room temperature (25 °C) in the mid-infrared range of 4000 to 450 cm^{-1} with a resolution of 4 cm^{-1}. Each spectrum was averaged over 32 scans to improve the signal-to-noise ratio [35]. Prior to each measurement, a background spectrum was recorded using a clean, dry ATR crystal and automatically subtracted using the instrument's Spectrum IR software (Spectrum IR, version 10.6.2.1159, Perkin Elmer). After each scan, the sample was removed, and the cell was cleaned with wipes soaked in propan-2-ol to prevent cross-contamination. The final IR spectra were recorded and analyzed using Spectrum IR software (Spectrum IR, version 10.6.2.1159, Perkin Elmer) to identify signal differences between samples.

2.6. Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Elemental concentrations were determined using an inductively coupled plasma mass spectrometer (ICP-MS) (Agilent 8800, Santa Clara, CA, USA), following the optimized method described by Potočnik et al. [36]. Samples were first decomposed in an UltraWAVE microwave decomposition system (MILESTONE Srl, Sorisole Italy), which allows for high-pressure, high-temperature digestion in a closed-vessel environment to ensure complete sample dissolution and minimize contamination. A known amount (0.08 g) of sample and 1 mL of 65% HNO_3 were added to a clean Teflon digestion vessel. The decomposition was performed as follows: samples were first heated to 240 °C for 20 min, maintained at 240 °C for 15 min and then allowed to cool to 40 °C. The maximum pressure was set to 100 bars. Nitrogen (N_2) was used as the loading gas at room temperature (25 °C) and pressure (25 bar). The decomposed samples were transferred into plastic vials and made up to 10 mL with Milli-Q water. In the case of the sediment at the bottom of the vial, the sample was filtered through a hydrophilic 0.45 µm filter (Sartorius, Germany) and diluted (1:5 and 1:10) with 5% HNO_3.

Two reference materials were also analyzed: tomato (SRM 1573a), peach (SRM 1547), and spinach leaves (SRM 1570a). The detection limits for the selected elements are as follows: Na (2 µg/g), Mg (0.5 µg/g), Al (5 µg/g), P (0.4 µg/g), S (2.2 µg /g), K (11 µg/g), Ca (22,000 ng/g), V (1 ng/g), Cr (9.5 ng/g), Mn (17 ng/g), Fe (600 ng /g), Co (1 ng/g), Ni (35 ng/g), Cu (25 ng/g), Zn (450 ng/g), As (3 ng/g), Se (5 ng/g), Rb (6 ng/g), Sr (130 ng/g), Mo (5 ng/g), Ag (2 ng/g), Cd (1.5 ng/g), Cs (0.6 ng /g), Ba (50 ng/g), Hg (0.7 ng/g), and Pb (15 ng/g). Replicate measurements showed good reproducibility, with relative standard deviations (RSD) generally below 5%, confirming the reliability of the ICP-MS data. An RSD of 5–10% was considered acceptable for elements present at concentrations near the detection limit.

2.7. Isotope Ratio Mass Spectrometry (IRMS)

The isotopic ratios of $^{13}C/^{12}C$, $^{15}N/^{14}N$ and $^{34}S/^{32}S$ values were determined using isotope ratio mass spectrometry (IRMS). The results were expressed as $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$. Measurements were made using a Vario IsoPrime100—Vario PYRO Cube (OH/CNS Pyrolyser/Elemental Analyzer; IsoPrime, Cheadle, Hulme, UK)). Samples were prepared by weighing a known amount of lyophilized sample (5 mg) into a tin capsule together with an equal amount of tungsten (VI) oxide (WO_3) as a combustion accelerator. The tin capsule was then sealed and placed into the elemental analyzer. International and laboratory reference materials with a known isotopic composition were used for quality control.

The results for carbon were normalized according to the following international reference substances: USGS91 (rice flour with a weight of 6.0 mg) with a value $\delta^{13}C$ of -28.28 ± 0.08‰ and USGS89 (porcine collagen with a weight of 6.0 mg) with a value $\delta^{13}C$ of -18.13 ± 0.11‰. Samples were normalized for nitrogen according to the following international reference materials: USGS91 with a value $\delta^{15}N$ of 1.78 ± 0.12‰, USGS43 (Indian human hair powder with a weight of 1.0 mg) with a value $\delta^{15}N$ of 8.44 ± 0.10‰ in USGS61 (1.0 mg weighed caffeine) with a $\delta^{15}N$ value of -2.87 ± 0.04‰. The laboratory reference material CRP-IAEA (casein protein, 2.8 mg) with a $\delta^{13}C$ value of -20.34 ± 0.09‰ and a $\delta^{15}N$ value of 5.62 ± 0.19‰ was used for the control. In the case of sulfur, the reference materials USGS43, with a $\delta^{34}S$ value of 10.46 ± 0.22‰, and USGS91, with a $\delta^{34}S$ value of -20.85 ± 0.72‰, were used to normalize the results. The control was the CRP-IAEA laboratory reference material with a $\delta^{34}S$ value of 4.18 ± 0.79‰. The measurement error for determining $\delta^{13}C$ and $\delta^{15}N$ values was ± 0.2‰ and ± 0.3‰ for $\delta^{34}S$.

2.8. Statistical Data Processing

All elemental and $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ data were statistically analyzed using Microsoft Excel, XLSTAT software package (ADDinsoft, New York, USA, 2019) and SIMCA-P (Soft Independent Modeling by Class Analogy; SIMCA 17.0.2, Sartorius Stedim Biotech, Umeå, Sweden) to identify differences between datasets. In XLSTAT, the Kruskal–Wallis test was performed for multiple groups based on geographical origin, and the Mann–Whitney test was applied for two groups based on the agricultural production method since the data did not follow a normal distribution. Additionally, discriminant analysis (DA) was used to enhance group differentiation. In SIMCA, Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) was used to reveal patterns and relationships within the data. Candidates for discriminant markers were chosen according to the Variable Importance in Projection (VIP) values derived from the OPLS-DA models. VIP values > 1 were significant and served as the cutoff for identifying the most relevant discriminant markers to differentiate between groups.

3. Results and Discussion

3.1. Paprika

3.1.1. Antioxidant Activity (AA) and Total Phenolic Compound (TPC) Content

Antioxidant activity values ranged from 2.36 ± 0.08 mg TE/g LV to 4.65 ± 0.07 mg TE/g LV, with the highest values recorded in P1 (4.65 ± 0.07 mg TE/g LV), P15 (4.60 ± 0.07 mg TE/g LV), and P2 (4.52 ± 0.05 mg TE/g LV). These samples represented non-organic sweet paprika from Hungary and Peru, indicating that these growing regions may provide favorable conditions for maintaining a high antioxidant capacity. Conversely, the lowest AA values were observed in P2 (2.87 ± 0.06 mg TE/g LV), P42 (2.43 ± 0.07 mg TE/g LV), and P16 (2.36 ± 0.08 mg TE/g LV), which included hot and sweet paprika samples from Spain, Serbia, and India.

The TPC content in paprika samples ranged from 7.5 ± 0.2 mg GA/g LV to 16.2 ± 0.2 mg GA/g LV, with substantial variation across different samples. The highest values were observed in P27 (16.2 ± 0.2 mg GA/g LV, fragmented paprika from Spain), P25 (15.4 ± 0.1 mg GA/g LV, organic sweet paprika from Spain), and P22 (15.0 ± 0.1 mg GA/g LV, organic smoked paprika from Spain). Notably, P27, which was in fragmented form rather than ground, exhibited the highest retention of phenolic compounds, which may be due to its reduced surface area exposure, minimizing oxidation and degradation. The lowest TPC levels were recorded in P42 (7.5 ± 0.2 mg GA/g LV, sweet paprika from Spain), P16 (8.1 ± 0.1 mg GA/g LV, hot paprika from India), and P39 (8.3 ± 0.2 mg GA/g LV, sweet paprika from Serbia). The antioxidant activity (AA) and total phenolic content (TPC) of ground paprika samples are presented in Table S3 of the Supplementary Material.

Despite the observable differences among the samples, no strong correlation was found between geographical origin, agricultural production method, paprika variety, and phenolic content. Likewise, no statistically significant difference was observed between organic and conventional paprika regarding antioxidant potential, suggesting that the agricultural production method alone does not substantially influence radical scavenging activity. Additionally, hot paprika varieties did not consistently exhibit higher antioxidant activity than sweet varieties, indicating that capsaicinoid content may not be the primary driver of antioxidant potential. Instead, it is possible that post-harvest processing techniques, drying conditions, and storage duration play a more dominant role in polyphenol retention and antioxidant activity, as these factors are known to influence polyphenol stability. These findings align with previous studies [6,37–39], which highlight the significant influence of environmental and processing factors on the bioactive compound profile of paprika.

3.1.2. Correlation Between Antioxidant Activity and Total Phenolic Content

A moderate correlation (R^2 = 0.555) was found in paprika samples, indicating that a higher TPC generally corresponded to higher AA. However, TPC values often exceeded AA values, suggesting that the Folin–Ciocalteu method might overestimate phenolic content due to interactions with non-phenolic compounds such as sugars and amino acids. Additionally, other compounds in paprika, including carotenoids, vitamins C and E, and capsaicinoids, may significantly contribute to antioxidant activity, influencing the observed correlation.

3.1.3. FTIR Results

Highly similar IR spectra were observed across all analyzed ground paprika samples, with some minor differences. For representative presentation, the IR spectra of non-organic sweet peppers from Spain (P8, P10, P38, P42, P45), along with crushed sweet peppers (P26) of the same origin and conventional production, are illustrated in Figure 1.

The spectral analysis identified key peaks in both the functional group (3600–1200 cm^{-1}) and fingerprint (1200–600 cm^{-1}) regions, detailed in Table S5 of the Supplementary Material. Notable peaks were observed at 3280, 2920, 1740, 1622, and 1030 cm^{-1}, with the most prominent at 1027 cm^{-1}. Minor peaks appeared at 1236, 1147, and 816 cm^{-1} [40,41].

A broad band (3600–3000 cm^{-1}) with a peak at 3280 cm^{-1} was linked to O–H stretching vibrations, likely from water or polyphenols [41,42]. A smaller band at 3010 cm^{-1} was only notable in samples P19, P41, P42, P44, and P45. Peaks at 2950–2800 cm^{-1} were tied to C-H stretching in CH_3 and CH_2 groups. Peaks at 2920 and 2850 cm^{-1} were assigned to CH_2 stretching vibrations [40], while a peak at 2110 cm^{-1} (C≡C stretching) may result from crystal-enhanced ATR-FTIR. Additionally, a weak band was observed above 3000 cm^{-1},

which could be attributed to aromatic C-H stretching or possibly to non-conjugated achene double bonds.

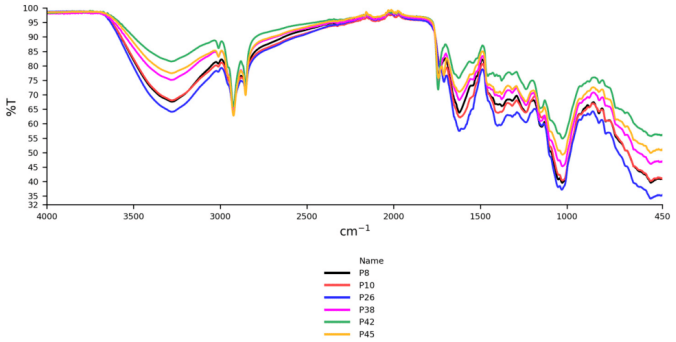


Figure 1. IR spectra of sweet pepper samples of Spanish origin (P8, P10, P26, P38, P42, P45).

The 1743 cm^{-1} peak was attributed to C=O stretching vibration, might be associated with oil resin components like capsanthin and capsorubin [43]. Its presence varied, with it being absent in several samples (e.g., P10, P11, P14), indicating possible adulteration. Samples that lacked this peak also exhibited a peak shift to 1710 cm^{-1}, which may correspond to capsaicin. These differences support previous findings linking genuine paprika to carotenoid peaks, which are often missing in altered samples [44].

The first derivative spectra for P8 and P10 (Figure 2) and comparisons with Galvin-King et al. [44] (Figure 3) show notable spectral differences, reinforcing variations in counterfeit samples.

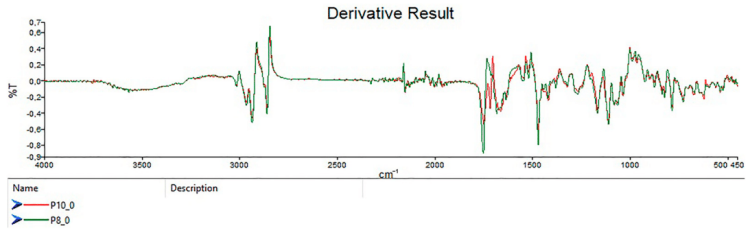


Figure 2. Derivatives of the IR spectra for sweet ground paprika samples from Spain P8 and P10.

In the fingerprint region, the dominant peak at 1027 cm^{-1} was consistent across samples, related to C-C vibrations and carbohydrate-related oscillations [37]. Peaks at 1236 and 1147 cm^{-1} were also found but were less pronounced in some samples (e.g., P10, P11). A minor peak at 816 cm^{-1}, likely from C-H or O-H deformations, was found in several samples [40].

In samples lacking the 1743 cm^{-1} peak, peaks at 1377 and 1400 cm^{-1} were observed, showing compositional differences. The 1377 cm^{-1} peak (symmetrical CH_3 deformation vibrations) was absent or weak in several samples, while the 1400 cm^{-1} peak, hypothetically linked to azo dyes like solar yellow, E110, was observed in these samples [40]. Azo dyes, though allowed in some foods, are banned in ground paprika due to their potential to mask quality issues.

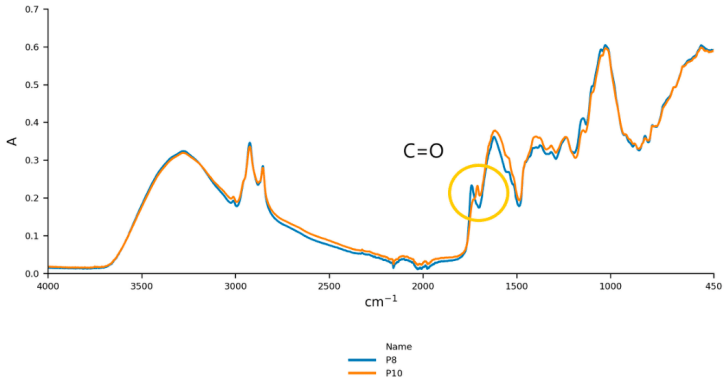


Figure 3. The peaks at 1743 cm^{-1} observed in our paprika samples (P8, P10) are similar to those reported in ground paprika by Galvin-King et al. [44], attributed to C=O stretching vibrations, as marked by the yellow circle.

Most samples with a 1400 cm^{-1} peak also lacked the 1743 cm^{-1} peak, suggesting synthetic dye use to replace natural pigments. The 1400 cm^{-1} peak may also signal use of leftover peppers post-resin extraction. For instance, P26 (crushed, not powdered) showed a strong 1400 cm^{-1} and weak 1377 cm^{-1} peak, possibly reflecting processing or storage effects. These hypotheses require further chemical confirmation.

A similarity analysis of paprika samples focusing on origin, variety, and production method (Table S6; Supplementary Materials) found strong correlations (0.996) in some pairs (e.g., P38–P6, P15–P35) and weaker ones (0.706) in others (e.g., P42–P26). Samples without the 1743 cm^{-1} peak showed high mutual similarity (0.920–0.991), while samples with this peak also clustered tightly (0.902–0.996), suggesting its critical role in sample grouping.

FTIR analysis showed that smoking, origin, and production method had little effect on overall composition. Differences in FTIR were minor, similarly to AA and TPC. The variability of paprika samples from different producers was comparable to that observed within samples from a single producer, suggesting that production processes and origin contribute minimally to the differences in paprika composition, aligning with Horn et al. [43].

3.1.4. Multi-Elemental Composition

The elemental composition of 45 ground paprika samples was categorized (Table 1) into macroelements (Na, Mg, Ca, P, S, K), microelements (Fe, Zn, Cu, Mn), and toxic elements (Hg, Cd, Pb, As).

In the paprika samples, the average elemental distribution (mg/kg) was K (26,707) > P (3795) > Mg (2571) > S (2229) > Ca (2204) > Na (414) > Fe (258) > Al (238) > Zn (20.8) > Mn (19.1) > Sr (17.4) > Rb (16.5) > Cu (11.0) > Ba (3.56) > Cr (1.91) > Ni (1.39) > V (0.45) > Co (0.29) > Pb (0.19) > As (0.11) > Cd (0.096) > Cs (0.071) > Se (0.065) > Ag (0.013) > Hg (0.0017). Similar patterns were found in previous studies on ground paprika [45].

The elemental composition of ground paprika revealed that Cr levels ranged from 0.16 to 5.74 mg/kg, exceeding the range reported by Garcia et al. [46] (0.19–0.65 mg/kg). Similarly, Al content was significantly higher (18–629 mg/kg) compared to the values reported by Lopez et al. [47] (12–43.6 mg/kg). Sweet peppers demonstrated higher levels of Fe (75.9–534 mg/kg; mean 272 mg/kg) than hot peppers (96.7–607 mg/kg; mean 232 mg/kg), aligning with findings by Palacios-Morillo et al. [45]. Potassium was the most abundant element, with average concentrations in sweet peppers (27.5 g/kg) slightly higher

than in hot peppers (25.7 g/kg), consistent with Hungarian paprika samples studied by Ördög et al. [48]. While Mn and Mg content showed minimal variation between sweet and hot peppers, our results indicated a higher Mo concentration in sweet peppers (448 μg/kg) compared to hot peppers (323 μg/kg), differing from the results found by Ördög et al. [48].

Table 1. Range of the concentrations of elements in paprika samples.

Elements	Paprika (mg/kg)
Macroelements	
Na	98.2–1540
Mg	1670–3310
P	2580–4720
S	1840–2780
K	17,400–34,300
Ca	854–3580
Microelements	
V	0.03–1.18
Cr	0.16–5.74
Mn	8.88–40.2
Fe	66.2–607
Co	0.05–1.18
Ni	0.13–3.84
Cu	7.71–13.8
Zn	13.6–32.5
Al	17.7–629
Se	0.006–0.17
Rb	5.59–41.1
Sr	3.87–33.4
Mo	0.07–1.97
Ag	0.001–0.09
Cs	0.01–0.20
Ba	0.62–7.88
Toxic elements	
Hg	0.0004–0.0032
Pb	0.01–0.54
As	0.02–0.18
Cd	0.028–0.318

Regarding toxic elements, lead (Pb) concentrations in ground paprika were below the EU limit of 0.60 mg/kg (Commission Regulation (EU) 2021/1317), with the highest concentration (0.537 mg/kg) in P26 (crushed sweet pepper from Spain) and the lowest (<0.015 mg/kg) in P23 (organic sweet pepper from Spain). Cadmium (Cd) levels ranged from 0.028 to 0.318 mg/kg, with some samples exceeding regulatory thresholds after applying conversion factors for dry weight. Arsenic was highest in P19 (0.183 mg/kg), while Hg content was negligible, with the maximum detected at 0.003 mg/kg in P24.

3.1.5. Stable Isotope Composition of Light Elements

The measured $\delta^{13}C$ values in paprika samples ranged from $-29.7 \pm 0.1‰$ to $-26.4 \pm 0.1‰$, consistent with the typical isotopic signature of C3 plants. All other samples fell within this range. The highest $\delta^{15}N$ value was found in sample P23 ($11.1 \pm 0.04‰$), while the lowest was in sample P29 ($1.8 \pm 0.2‰$). The $\delta^{34}S$ values varied between $2.3 \pm 0.2‰$ and $11.1 \pm 0.2‰$, considering paprika from various geographical origins and agricultural practices. Compared with previous studies, Mahne Opatić et al. [49] reported a

δ^{34}S range of 1.3‰ to 15.0‰ for sweet peppers from multiple countries, aligning with the present study findings (δ^{34}S range: 3.0–11.1‰). Their reported averages for δ^{34}S were Slovenian (2.9‰), Spanish (5.8‰), Italian (6.4‰), Moroccan (8.4‰), and Greek (12.2‰). The δ^{13}C values for the same countries were: −27.5‰ (Slovenia), −27.7‰ (Spain), −27.4‰ (Italy), −26.9‰ (Morocco), and −25.8‰ (Greece). For δ^{15}N: Slovenian (6.0‰), Spanish (3.1‰), Italian (−4.1‰), Moroccan (1.9‰), and Greek (2.4‰). Spanish paprika, including both organic and non-organic samples, produced comparable averages: δ^{13}C (−28.1 ± 0.03‰), δ^{15}N (4.8 ± 0.1‰), and δ^{34}S (8.3 ± 0.1‰).

3.1.6. Differentiating Samples According to the Country of Origin

These results supported the use of multivariate approaches for origin discrimination. However, due to limited sample availability, China and Peru were excluded from further modeling. Discriminant analysis (DA) was subsequently used to assess the geographical origin of paprika samples from Hungary, Serbia, and Spain, based on isotopic ratios (δ^{13}C, δ^{15}N, δ^{34}S) and elemental composition.

Thirty samples were analyzed, including sweet and spicy varieties produced via organic and conventional methods. Spanish samples formed the most distinct and well-separated group, followed by Serbian samples. In contrast, Hungarian samples exhibited some overlap with the Spanish group, with their centroids positioned close together (Figure 4). Twelve isotopic and elemental variables—δ^{34}S, Mg, Sr, Cs, Rb, V, δ^{13}C, Fe, Al, P, S, and Ba—were identified as the most influential for origin classification, as shown by variables with VIP scores > 1 (Figure 4). These results align with earlier studies, including those of Brunner et al. [30] and Fiamegos et al. [50,51], who also demonstrated the effectiveness of these markers in paprika provenance analysis. The obtained OPLS-DA resulted in two predictive and one orthogonal component (2 + 1), producing an $R^2X = 0.476$, $R^2Y = 0.567$, and $Q^2 = 0.226$. Classification metrics (sensitivity, specificity, precision, F1 score) were calculated for each class individually and summarized using macro average. The F1 score rate, obtained by internal cross-validation, was 83.6%, sensitivity was 80.0%, specificity was 87.5%, precision was 96.0%, and accuracy was 90.0%.

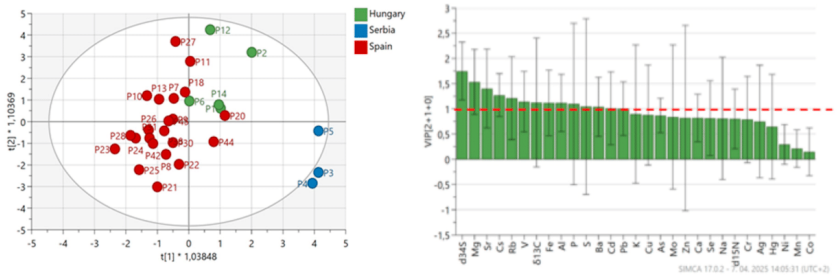


Figure 4. OPLS-DA models differentiating geographical origins (Hungary, Serbia, Spain) based on isotopic ratios and elemental composition: (**a**) score plot illustrate sample clustering by country—Hungary (green), Serbia (blue), and Spain (red)—with a 95% confidence interval ellipse; (**b**) Variable Importance in Projection (VIP) plot highlight the most influential variables (VIP > 1, red dashed line) for group discrimination.

Spanish samples were characterized by distinctively higher δ^{13}C and Sr values, suggesting differences in climate (e.g., aridity and sunlight intensity) and soil mineral composition, likely reflecting the more calcareous or marine-influenced soils found in parts

of Spain. Higher δ^{34}S values may also indicate the use of sulfur-rich fertilizers or the influence of proximity to marine environments. Serbian samples exhibited the highest δ^{15}N values (5.2‰) and S content (2465 mg/kg), likely due to fertilizer use, while Hungarian samples displayed intermediate values for most variables. Elements such as Mg, Fe, Al, and P—which are present to some extent in samples from both Hungary and Spain due to similar soil types and farming techniques [48]—still contributed to the separation when evaluated in combination with other variables. This phenomenon is supported by studies showing that agricultural practices and soil management can have a greater impact on the elemental composition of crops than geographic proximity alone [30,50]. Trace elements like Cs, Rb, and Ba, which are often influenced by the underlying geology and long-term land use, provided further resolution. Despite some misclassification among Hungarian samples, the model exhibited strong discriminatory power, with statistically significant separation by origin. Serbian and Spanish samples were classified with perfect accuracy (100%), whereas Hungarian samples showed lower accuracy, with only 40% correctly classified.

Closer inspection of the score plot revealed that several Spanish samples (P11, P18, P20, and P27) clustered near the Hungarian group. While P20 appeared typical, samples P11, P18, and P27 displayed unusual chemical and spectral characteristics. FTIR analysis showed that these samples lacked the characteristic 1743 cm^{-1} absorption band associated with paprika oleoresins, suggesting the presence of potential residual plant matter or unauthorized coloring agents. These anomalies may indicate low-quality or adulterated material, which could distort the chemical profiles and obscure true geographic signals.

In contrast, Serbia's more isolated agricultural context likely preserved a distinct chemical fingerprint, supporting its excellent classification. Lastly, since all paprika samples were commercially sourced without certified origin documentation, the possibility of mislabeling or origin fraud cannot be excluded and should be considered when interpreting these findings. Further, model evaluation was conducted with full consideration of the sample size imbalance, and the results were interpreted cautiously. This limitation highlights the need for more balanced and representative sampling in future studies to ensure greater reliability and generalizability of the classification models.

3.1.7. Differentiating Samples According to the Agricultural Production Practice

A Mann–Whitney test confirmed statistically significant differences ($p < 0.05$) in elemental composition and isotope ratios between organic (n = 5) and conventional (n = 37) paprika samples. Conventional samples exhibited higher concentrations of Na, Al, V, Cr, Fe, Rb, Cs, Ba, and Pb, likely due to metal-based fertilizers, pesticides, and environmental contamination. In contrast, organic samples were characterized by elevated Zn, δ^{15}N, and δ^{34}S values, reflecting the use of organic fertilizers.

Toxic element levels, particularly As and Pb, were significantly higher in conventional samples, with Pb concentrations averaging 213 µg/kg in conventional samples compared to 79 µg/kg in organic samples. Box plots of concentrations of elements (Na, Al, V, Cr) and values δ^{15}N and δ^{34}S in samples can be seen in Figure 5, while box plots of other elements—Fe, Zn, As, Rb, Ba, Pb, and Cs—can be found in the Supplementary Materials (Figure S1).

These findings align with those of Fiamegos et al. [50], who observed similar trends in paprika using ED-XRF. Their study found organic samples with higher Mg, K, S, P, Cl, Zn, and Br concentrations, while conventional samples contained higher Fe, Mn, Cr, Ba, and Rb levels. The isotopic marker δ^{15}N emerged as a reliable indicator of organic farming, with all organic paprika samples exceeding 7‰, consistent with findings by Schmidt et al. and Flores et al. [52,53]. These studies demonstrated that organic fertilizers enriched in ^{15}N have higher δ^{15}N values than synthetic fertilizers, which are nearer to zero.

In contrast, δ^{13}C showed limited utility for distinguishing farming practices, as differences were statistically insignificant.

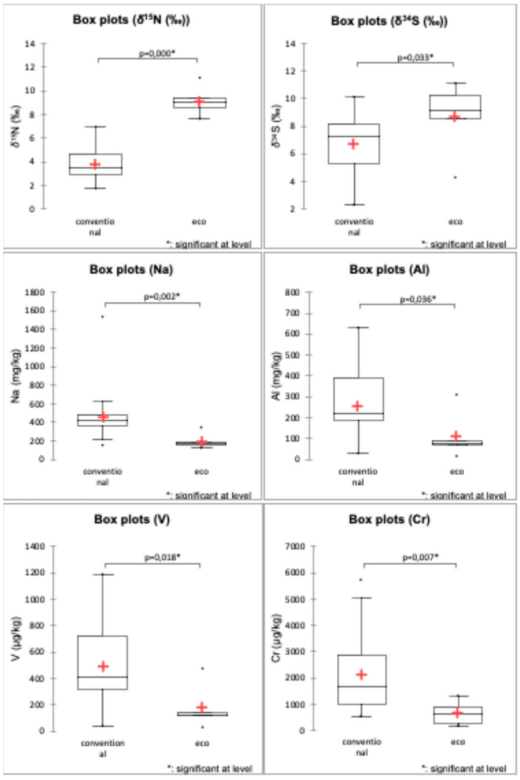


Figure 5. Element concentrations (Na, Al, V, Cr), δ^{15}N, and δ^{34}S values in paprika samples according to the agricultural production method, where statistical differences were observed. The red "+" symbol indicates the mean value for each group.

Perez-Lopez et al. [54] show how agricultural practices significantly influenced the mineral concentrations of paprika grown in greenhouses under controlled conditions, thereby eliminating climatic variability as a factor. They observed higher concentrations of most minerals in organic paprika compared to integrated and conventional products. While their findings partially align with this study, there are notable differences. Their study showed higher Fe concentrations in conventional paprika and no statistically significant differences in Cu or Ca levels between organic and conventional samples. These discrepancies may stem from differences in experimental conditions, such as the greenhouse-controlled environments used in their study versus the commercially sourced samples of various origins in this study, as well as variations in fertilization practices or regional soil compositions.

3.2. Cinnamon

3.2.1. Antioxidant Activity (AA) and Total Phenolic Compound (TPC) Content

Cinnamon samples exhibited significantly higher antioxidant activity than paprika samples, with AA values ranging from 53 ± 1 mg TE/g LV to 404 ± 1 mg TE/g LV. The highest AA values were recorded in C24 (404 ± 1 mg TE/g LV, organic ground cinnamon of unknown geographical origin and C21 (341 ± 1 mg TE/g LV, ground cassia cinnamon from Indonesia). In contrast, the lowest AA values were found in C5 (55 ± 1 mg TE/g LV), C23 (53 ± 1 mg TE/g LV), and C11 (56 ± 1 mg TE/g LV), all of which were organic Ceylon cinnamon samples from Sri Lanka.

Cinnamon samples contained significantly higher phenolic content than paprika samples, with TPC values ranging from 33 ± 1 mg GA/g LV to 254 ± 1 mg GA/g LV. The highest levels were found in C24 (254 ± 1 mg GA/g LV, organic ground cinnamon) and C21 (247 ± 1 mg GA/g LV, cassia cinnamon from Indonesia), while the lowest were recorded in C5 (33 ± 1 mg GA/g LV, organic Ceylon cinnamon from Sri Lanka), C23 (40 ± 1 mg GA/g LV, organic Ceylon cinnamon from Sri Lanka), and C11 (40 ± 0.2 mg GA/g LV, Ceylon cinnamon from Sri Lanka). The antioxidant activity (AA) and total phenolic content (TPC) of cinnamon samples are presented in Table S4 of the Supplementary Material.

The botanical species of cinnamon significantly influenced antioxidant activity (AA) and phenolic content, with cassia cinnamon consistently exhibiting higher values than Ceylon cinnamon. The highest AA levels were observed in cassia samples from Indonesia and Vietnam (C21, C12, C33), whereas the lowest were found in Ceylon cinnamon from Sri Lanka and Madagascar. Similarly, cassia cinnamon contained a greater number of phenolic compounds compared to Ceylon cinnamon. It should be noted that the DPPH assay is not specific to phenolic compounds, and the observed antioxidant activity may also reflect the presence of non-phenolic reducing substances such as ascorbic acid or Maillard reaction products. In the case of cassia cinnamon, the high antioxidant activity values may therefore not be solely attributed to phenolic content. Within the Ceylon group, samples from Madagascar demonstrated higher total phenolic content (TPC) than those from Sri Lanka, suggesting that environmental conditions and genetic variation among cultivars play a role in polyphenol composition.

Form of storage also proved critical; cinnamon sticks displayed higher antioxidant activity and phenolic content than ground cinnamon of the same botanical species and origin, likely due to reduced oxidation and better preservation of bioactive compounds during storage. Furthermore, cinnamon from conventional farming exhibited higher AA and TPC values than organic samples, particularly cassia cinnamon, where non-organic samples showed a markedly increased radical scavenging capacity. These trends are consistent with the findings of Lv et al. [42], who attributed the higher antioxidant potential and polyphenol levels in conventional cinnamon to differences in the concentration of free and bound soluble phenolic compounds.

3.2.2. Correlation Between Antioxidant Activity and Total Phenolic Content

Cinnamon samples exhibited a strong correlation (R^2 = 0.875) between AA and TPC, confirming that phenolic compounds are major contributors to cinnamon's antioxidant capacity. Most cinnamon samples showed higher AA values than TPC, except for C12 and C10. The strong relationship suggests that the phenolic compounds measured by the Folin–Ciocalteu method play a crucial role in cinnamon's antioxidant potential. However, bioactive compounds, like proanthocyanidins and trans-cinnamaldehyde, indicate that not all antioxidant activity is derived solely from TPC. Similar findings were reported by Lv et al. [42], who found an even stronger correlation (R^2 = 0.979) between TPC and AA in cinnamon.

3.2.3. FTIR Results

FTIR revealed only minor differences between the cinnamon samples. The IR spectra of non-organic Ceylon ground cinnamon from Sri Lanka (C9, C10, C11) and Madagascar (C13, C16) are shown in Figure 6.

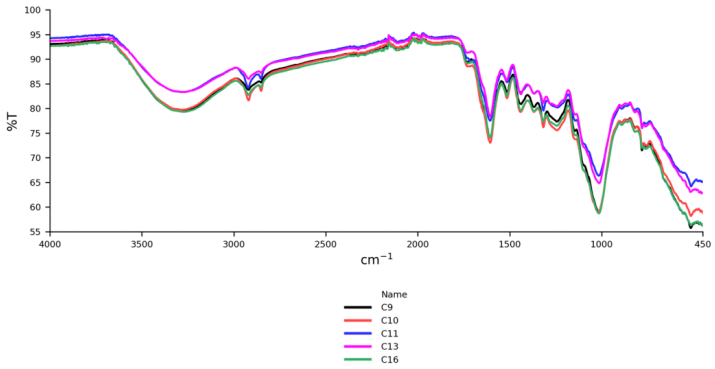


Figure 6. IR spectra of samples of Ceylon cinnamon from Sri Lanka (C9, C10, C11) and Madagascar (C13, C16).

Prominent spectral peaks appeared at 3280, 2920, 1605, and 1015 cm^{-1}, with the peak at 1015 cm^{-1} being most intense. Minor peaks were noted at 2110, 1516, 1440, and 780–750 cm^{-1} (Table S7; Supplementary Materials). These indicate chemical composition and functional groups in the samples.

A broad band at 3600–3000 cm^{-1}, peaking at 3280 cm^{-1}, was linked to O–H stretching vibrations, possibly through water or phenolic compounds like catechin or caffeic acid [35,55]. It could also reflect alcohols (e.g., eugenol) or proteins, as noted by Lixourgioti et al. [32]. This peak was stronger in Ceylon cinnamon, indicating higher phenol and eugenol content, while cassia had an absent or weak peak, suggesting a lower content of phenols and eugenol.

Peaks at 2920 and 2850 cm^{-1} corresponded to asymmetric and symmetric C–H stretching vibrations in CH_2 groups, likely from lipids or compounds like cinnamaldehyde and eugenol. These were less evident in some samples (e.g., C22, C38), suggesting differences in lipid content or sample preparation.

A strong peak at 1015 cm^{-1}, seen across all samples, indicated C–O stretching vibrations in esters and phenolics. The 1605 cm^{-1} peak reflected C=C stretching vibrations in cinnamaldehyde, a major volatile component of cinnamon bark. Some variation in the 1730 cm^{-1} peak (C=O stretching of aldehydes) was observed, with slight shifts in samples (1715 cm^{-1}; C10, C11) indicating differences in cinnamaldehyde or other carbonyl compounds. Samples C7 and C24 lacked the peak at 1730 cm^{-1}, possibly due to origin or processing method.

3.2.4. Ceylon Cinnamon vs. Cassia

Peaks between 780 and 750 cm^{-1}, representing out-of-plane C–H bending vibrations, were particularly significant for distinguishing between Ceylon cinnamon (*C. verum*) and cassia (*C. cassia*). In Figure 7, the IR spectra of cassia (C33) and Ceylon cinnamon (C5) highlight the differences: cassia samples exhibited a well-defined peak at 750 cm^{-1}, while Ceylon cinnamon samples displayed a pronounced peak at 780 cm^{-1}. These findings are consistent with previous studies by Li et al. [56] and Yasmin et al. [57], which noted

the association of the 750 cm^{-1} peak with coumarin, a compound prevalent in cassia but minimal in Ceylon cinnamon. Thus, this distinct band may serve as a reliable spectral marker for the identification of cassia cinnamon.



Figure 7. Differentiation of the IR spectrum of cassia (C33) and Ceylon cinnamon from Sri Lanka (C5) at peaks 780 and 750 cm^{-1}. The green ellipse emphasizes the spectral region where Ceylon cinnamon exhibits a pronounced peak only at 780 cm^{-1}, with no detectable peak at 750 cm^{-1}. In contrast, cassia displays distinct peaks at both 750 cm^{-1} and 780 cm^{-1}. The absence of a peak at 750 cm^{-1} in Ceylon cinnamon serves as a key spectral marker distinguishing it from cassia.

3.2.5. Organic vs. Non-Organic Cassia

FTIR revealed notable differences in the fingerprint region between organic and non-organic cassia. Organic samples from Indonesia (C6) and Vietnam (C33) showed distinct peaks at 750 cm^{-1} and 780 cm^{-1}, indicating stable phenolic and volatile compound compositions. Non-organic samples from Indonesia (C21, C41) showed a clear peak at 750 cm^{-1} but a weaker one at 779 cm^{-1}. Sample C12, also non-organic cassia, had a clear peak at 762 cm^{-1}, possibly due to regional variations. Li et al. [56] highlighted that soil, climate, and altitude affect cassia's essential oil content. Oil cells, the primary sites for synthesizing and accumulating essential oils, tend to exhibit stable compositions, but regional factors can still introduce variability. Still, all non-organic samples of cassia (C12, C21, and C41) shared a weak 779 cm^{-1} peak, setting them apart from organic ones. These findings suggest that organic cassia consistently shows a strong 780 cm^{-1} peak, while non-organic types do not, supporting FTIR's use in distinguishing them.

3.2.6. Organic vs. Non-Organic Ceylon Cinnamon

Silva Bruni et al. [58] analyzed mid-infrared (MIR) spectra of true (Ceylon) cinnamon and found a key feature for differentiation between organic and non-organic cinnamon samples above 2600 cm^{-1}, where O–H stretching and C–H vibrations linked to eugenol and phenolics were evident. Organic cinnamon showed higher transmittance (lower absorbance), indicating lower phenolic content, while non-organic samples showed the opposite (Figure 8). These findings matched this study's results for cassia and Ceylon cinnamon, especially Sri Lankan samples.

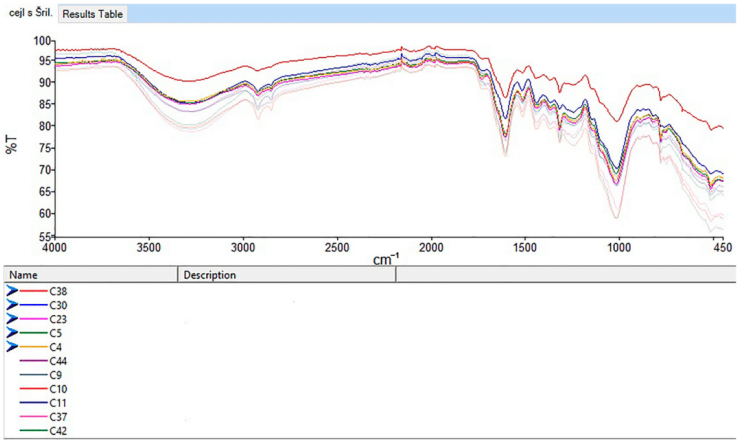


Figure 8. Comparison of IR spectra between organic (C38, C30, C23, C5, C4—indicated by a blue arrow) and non-organic (C44, C9, C10, C11, C37, C42) Ceylon cinnamon samples from Sri Lanka.

For Sri Lankan Ceylon cinnamon, organic samples showed higher transmittance and thus lower phenolic content, which was confirmed by AA and TPC results. In contrast, Ceylon cinnamon from Madagascar showed inconsistencies: sample C16 (non-organic) resembled organic patterns, while C15 (organic) mirrored non-organic traits (Figure 9). This variation may stem from geography, environment, or processing.

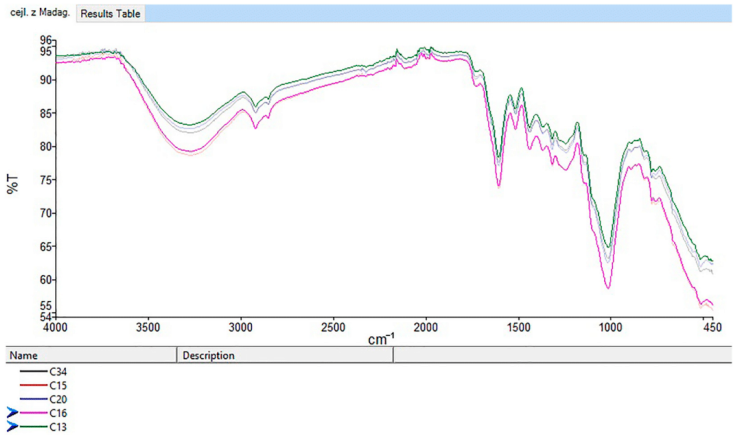


Figure 9. Comparison of IR spectra between organic (C15, C20, C34) and non-organic (C13, C16—indicated by a blue arrow) Ceylon cinnamon samples from Madagascar.

Cassia samples showed higher AA results and a higher phenolic content than Ceylon cinnamon from Sri Lanka, with lower IR transmittance (e.g., C6, C33 vs. C5, C23). Notably, Madagascar Ceylon samples (C15, C20) had similar transmittance values to cassia, indicating possible compositional overlap (Figure 10).

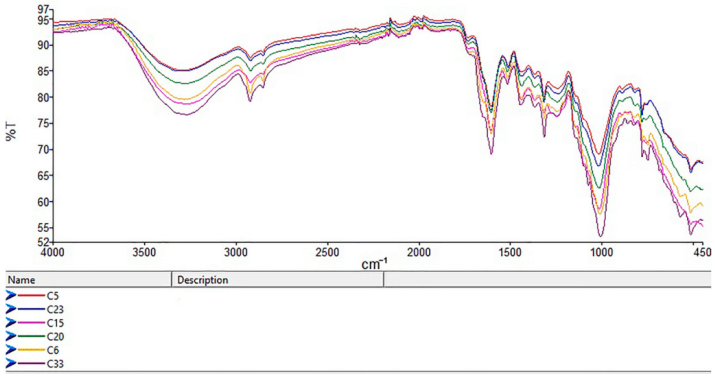


Figure 10. IR spectra of organic samples of cassia (C6, C33) and Ceylon cinnamon from Sri Lanka (C5, C23) and Madagascar (C15, C20).

A similarity analysis among all cinnamon samples with declared species, geographical origin, and production methods showed high correlations (0.94–0.98; Table S8; Supplementary Materials) within the following groups: organic Sri Lanka (C4, C5, C23, C30, C38) and organic Ceylon cinnamon from Madagascar (C15, C20, C34). The strongest correlation was 0.988 (C34 and C20). Non-organic Ceylon samples from Madagascar (C13, C16) also showed strong correlations (0.97–0.98) with other Ceylon cinnamon, despite spectral intensity differences in peaks at 780 and 760 cm^{-1}.

Lower values were noted for C37 (0.87–0.93) and C30 (0.91–0.93). Sample C41 showed notably lower similarity (0.76–0.86), indicating significant compositional differences, possibly due to species variation (e.g., *C. loureirii* or *C. burmannii*). Cassia samples showed lower correlations with Ceylon cinnamon (0.885–0.928), reflecting species-based differences. High similarity was found within organic cassia from Vietnam (C33) and Indonesia (C6) (0.965) and non-organic cassia from Indonesia (C12, C21) (0.977). C41 again showed lower correlations (0.831–0.848), reinforcing its distinct profile. These findings align with prior research [56], highlighting the influence of both region and species on cassia's chemical profile.

Overall, FTIR spectra showed strong similarities among cinnamon samples of the same species and production method, indicating stable composition. However, slight differences between organic and non-organic cassia (correlation values: 0.913–0.923) suggest variations in phenolic and volatile compounds. These findings align with AA and TPC analyses, highlighting the influence of species, origin, and agricultural practices on cinnamon's chemical composition.

3.2.7. Multi-Elemental Composition

The elemental composition of 46 cinnamon samples was categorized (Table 2) into macroelements (Na, Mg, Ca, P, S, K), microelements (Fe, Zn, Cu, Mn), and toxic elements (Hg, Cd, Pb, As).

The average distribution of elements in cinnamon samples, from highest to lowest concentration (mg/kg), was as follows: Ca (12,205) > K (7059) > S (1143) > Mg (833) > P (611) > Al (470) > Na (409) > Fe (331) > Mn (193) > Sr (87) > Ba (80) > Rb (22) > Zn (17) > Cu (6.5) > Cr (1.3) > Ni (0.85) > V (0.67) > Pb (0.61) > Cd (0.24) > Cs (0.23) > Co (0.18) > Mo (0.10) > As (0.042) > Se (0.039) > Hg (0.0095) > Ag (0.0032). These values are consistent with previous research [59,60], which identified calcium as the predominant element in cinnamon.

Table 2. Range of the concentrations of elements in cinnamon samples.

Elements	Cinnamon (mg/kg)
Macroelements	
Na	6–1760
Mg	517–1440
P	327–1200
S	638–3400
K	4420–15,500
Ca	6520–35,600
Microelements	
V	0.03–3.31
Cr	0.05–4.68
Mn	21.3–598
Fe	15.4–1680
Co	0.02–0.81
Ni	0.08–6.45
Cu	2.12–12.3
Zn	5.42–27.1
Al	23.2–2390
Se	0.003–0.14
Rb	7.62–39.2
Sr	32.3–256
Mo	0.01–0.27
Ag	0.001–0.02
Cs	0.03–0.89
Ba	22.5–152
Toxic elements	
Hg	0.002–0.062
Pb	0.021–4.01
As	0.01–0.25
Cd	0.05–0.70

The elemental composition displayed significant variation. Copper levels ranged from 2.1 to 12.3 mg/kg, lower than the range reported by Goncalves et al. [61] (7.2–21.9 mg/kg). Zinc concentrations (5.4–27.1 mg/kg) were consistent with those observed by Goncalves et al. [61] and Krejpcio et al. [62]. Iron levels (15.4–1680 mg/kg) were substantially lower than those of Singh and Garg [63], who reported 2390 mg/kg for true cinnamon. Similarly, Cr content ranged from 0.1 to 4.7 mg/kg, aligning with the results of Garcia et al. [46] but lower than those obtained by Özcan and Akbulut [60], who detected 7.89 mg/kg in true cinnamon.

Toxic element analysis showed that Pb was highest (4.01 mg/kg) in C25 (ground cinnamon from India), exceeding the EU limit for bark spices (2.00 mg/kg). Cadmium levels ranged from 0.049 to 0.701 mg/kg, with the highest detected levels in C39 (cinnamon sticks of unknown origin). Arsenic ranged from 0.010 to 0.248 mg/kg, while Hg content remained minimal (0.002–0.062 mg/kg). Unlike ground paprika, some cinnamon samples exceeded regulatory Pb limits, suggesting contamination, likely from environmental exposure. Paprika samples displayed higher K and Fe levels, while cinnamon contained higher concentrations of toxic elements, particularly lead and cadmium. This variability is attributed to differences in soil composition, geographic origin, and agricultural practices, as noted in previous studies [61–64]. Nevertheless, the hazard index (HI), representing the combined non-carcinogenic effects of all toxic elements, did not indicate a potential health risk for cinnamon consumers [65].

3.2.8. Stable Isotope Composition of Light Elements

Cinnamon samples exhibited $\delta^{13}C$ values from -32.6 ± 0.1‰ to -28.9 ± 0.1‰, confirming its classification as a C3 plant. The highest $\delta^{15}N$ value was 7.1 ± 0.1‰, observed in C44, and the lowest was 0.3 ± 0.2‰, found in C9. $\delta^{34}S$ values ranged from -0.9 ± 0.1‰ to 19.1 ± 0.1‰. Although the stable isotope composition of carbon (C), nitrogen (N), and sulfur (S) in cinnamon as a spice has not been previously reported, Sewenig et al. [66] analyzed cinnamon oil and found $\delta^{13}C$ values ranging from -27.1‰ to -25.9‰ for Ceylon cinnamon oils and -26.4‰ to -24.2‰ for cassia oils. Our results support this differentiation.

Organic cassia cinnamon samples had $\delta^{13}C$ values from -29.9‰ to -29.8‰, while conventional samples ranged from -29.6‰ to -29.3‰. The mean $\delta^{13}C$ value for cassia was -29.5‰. Ceylon cinnamon showed greater variation between organic and non-organic samples, ranging from -32.6‰ to -30.1‰. The mean $\delta^{13}C$ value for Ceylon cinnamon was -31.4‰, with samples from Madagascar averaging -32.3‰ and those from Sri Lanka -30.9‰. Samples C37 and C30 were excluded due to discrepancies.

Regarding $\delta^{15}N$, cassia samples from organic production showed values between 2.0‰ and 4.0‰ (mean 3.0‰), while conventional samples ranged from 1.4‰ to 2.1‰ (mean 1.8‰). The trend was inconsistent for Ceylon cinnamon, with overlapping ranges for organic and conventional samples. Cinnamon $\delta^{34}S$ values also varied significantly. Cassia from organic production ranged from 4.9‰ to 5.1‰ (mean 5.0‰), while conventional samples ranged from 3.9‰ to 5.7‰ (mean 4.8‰). Ceylon cinnamon showed wider variations, particularly in Madagascar, where both organic and conventional samples had high $\delta^{34}S$ values (18.3‰ and 18.1‰, respectively).

3.2.9. Differentiating Samples According to the Country of Origin

DA was conducted to assess the geographical origin of cinnamon samples from Sri Lanka, Indonesia, and Madagascar, following the exclusion of Vietnam due to limited sample availability. Twenty-seven samples were analyzed, including Ceylon and cassia cinnamon, sourced from conventional and organic production. Indonesian and Madagascan samples formed well-separated groups, while Sri Lankan samples exhibited some overlap, primarily with Madagascan samples (Figure 11). The differentiation of cinnamon samples by origin was primarily driven by fourteen isotopic and elemental variables (Rb, Cu, Ca, Se, $\delta^{13}C$, Na, $\delta^{34}S$, Cs, Mn, $\delta^{15}N$, Al, V, Cd, and Fe) with VIP scores > 1 (Figure 11). These findings partially align with those of Goncalves et al. [61], who identified significant variability in Fe and Al concentrations among cinnamon samples marketed in Portugal. However, their study did not consider geographic or environmental factors, focusing on quality control during production, transport, and storage.

Obtained OPLS-DA comprised two predictive and one orthogonal component (2 + 1), producing an $R^2X = 0.663$, $R^2Y = 0.699$, and $Q^2 = 0.498$. Classification metrics (sensitivity, specificity, precision, F1 score) were calculated for each class individually and summarized using macro average. The F1 score rate, obtained by internal cross-validation, was 88.5%, sensitivity was 92.9%, specificity was 95.7%, precision was 86.8%, and accuracy was 88.9%. Indonesian and Madagascan samples were classified with 100% accuracy, whereas Sri Lankan samples were correctly classified in 78.6% of cases.

Isotopic markers like $\delta^{13}C$ and $\delta^{15}N$ are influenced by climatic conditions and nitrogen sources, respectively, helping to distinguish regions with different rainfall patterns, sunlight exposure, and fertilization methods. $\delta^{34}S$ values can signal the use of sulfate-based fertilizers or proximity to marine environments. For example, Madagascar samples exhibit the highest $\delta^{34}S$ values, probably indicating the influence of the marine environment, while $\delta^{15}N$ values were the highest in Sri Lankan samples, probably influenced by production practices. Elemental markers such as Rb, Cs, and Al are indicative of the local geology, with

higher concentrations being observed in the Madagascar sample, while Fe, Cu, and Mn reflect micronutrient availability in soils, which is the lowest in Indonesia. Macronutrients like Ca, Na, and Se also vary depending on soil chemistry, irrigation water composition, and plant uptake efficiency.

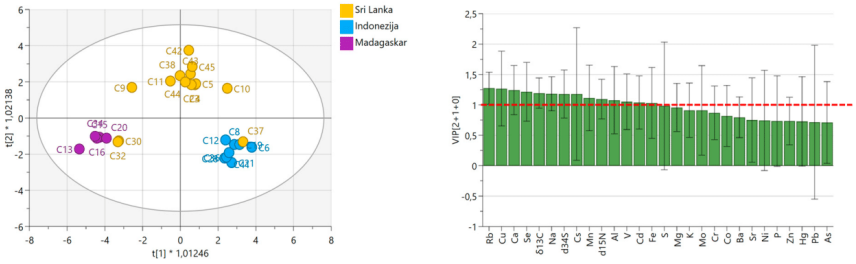


Figure 11. OPLS-DA models differentiating geographical origins (Sri Lanka, Indonesia, Madagascar) based on isotopic ratios and elemental composition. Score plots (**left**) illustrate sample clustering by country: Sri Lanka (yellow), Indonesia (blue), and Madagascar (purple), with the 95% confidence interval ellipse. Variable Importance in Projection (VIP) plots (**right**) highlight the most influential variables (VIP > 1, red dashed line) for group discrimination.

Misclassifications involved assigning Sri Lankan samples as Madagascar (e.g., C30) or Indonesia (e.g., C37). FTIR analysis revealed that sample C37, labeled non-organic Ceylon cinnamon from Sri Lanka, deviated markedly from typical Ceylon profiles. It exhibited a weak peak at 780 cm^{-1} and a strong peak at 750 cm^{-1}, a pattern characteristic of cassia cinnamon (Figure 8). Antioxidant profiling supported this observation, showing elevated phenolic content consistent with cassia, suggesting possible adulteration or mislabeling.

Sample C30, an organic Sri Lankan cinnamon, displayed weak peaks at 780 cm^{-1} and 750 cm^{-1}, resembling the spectral profile of Madagascan samples. This similarity could be attributed to divergent growing conditions, storage or handling issues, analytical variation, or incorrect origin labeling, complicating its classification. These results help explain the observed misclassifications. The cassia-like characteristics of C37 likely led to its misclassification as Indonesian cinnamon, while the overlap between C30 and Madagascan profiles contributed to its incorrect assignment. Environmental factors and the commercial sourcing of uncertified samples may have further contributed to these overlaps.

3.2.10. Differentiating Samples According to the Agricultural Production Practice

OPLS-DA effectively differentiated organic and conventional cinnamon production methods across various origins and species. Figure 12 illustrates the separation, with organic (eco) and conventional samples grouped within a 95% confidence ellipse. Key discriminating variables (VIP > 1), displayed above the red dashed line, include Ba, $\delta^{34}S$, Cu, Rb, $\delta^{13}C$, As, Mg, Co, Na, P, and S. Obtained OPLS-DA resulted in one predictive and two orthogonal components (1 + 2), producing an $R^2X = 0.483$, $R^2Y = 0.734$, and $Q^2 = 0.475$. The F1 score rate obtained by internal cross-validation was 94.1%, sensitivity was 94.1%, specificity was 94.7%, precision was 94.1%, and accuracy was 94.4%.

While most samples were correctly classified, discrepancies emerged. Sample C16, labeled as conventional, aligned more closely with organic cinnamon, particularly Ceylon cinnamon from Madagascar (correlation: 0.971), suggesting possible mislabeling or a transition to organic production. FTIR analysis supports this assumption (Figure 9).

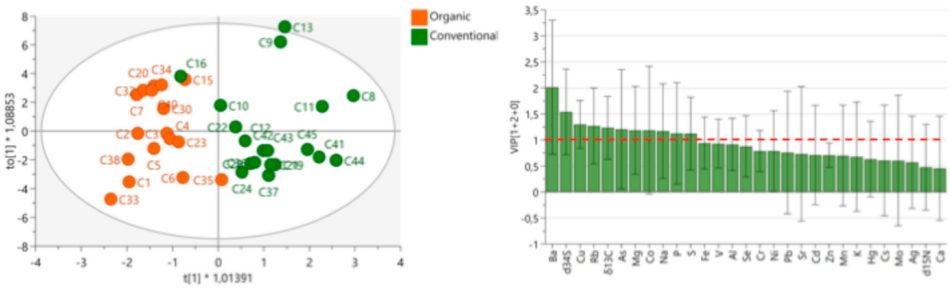


Figure 12. OPLS-DA models showing the differentiation between conventional (green) and organic (eco; orange) cinnamon samples based on isotopic ratios and elemental composition. The 95% confidence interval ellipse is shown. Variable Importance in Projection (VIP) plots (below) highlight the most influential variables (VIP > 1, indicated by the red dashed line) for group discrimination.

To confirm significant differences between organic and conventional samples, we applied the Mann–Whitney test to 17 conventional and 19 organic cinnamon samples, further validating the separation based on elemental and isotopic profiles. The Mann–Whitney test identified significant differences ($p < 0.05$) in δ^{34}S, P, and Ba between organic and conventional cinnamon (Figure 13).

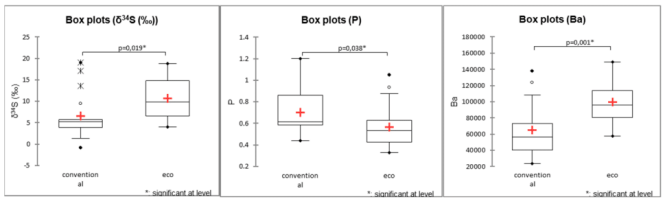


Figure 13. Box plots of δ^{34}S, P, and Ba concentrations in cinnamon samples by agricultural production method. The red "+" symbol indicates the mean value for each group.

Organic cinnamon production shows statistically significantly higher average concentrations of Ba (99.6 mg/kg) and δ^{34}S values (10.7‰), while conventional cinnamon production shows higher average concentrations of P (701 mg/kg), likely due to P-rich fertilizers. Similar trends were observed in paprika, where δ^{34}S helped differentiate organic and conventional production, with organic samples showing higher δ^{34}S values than conventional ones. This contrasts with the findings of Sinkovič et al. [67] in chicory, where organically produced samples exhibited lower δ^{34}S values compared to conventionally fertilized ones. Schmidt et al. [52] reported δ^{34}S as more indicative of origin than fertilizer type. Barium content also differed geographically (Sri Lanka—Vietnam; Indonesia—Vietnam), while P remained consistent, reinforcing its potential as a marker of production method. While δ^{34}S can provide valuable insights for distinguishing farming practices, its strong sensitivity to geographic origin and environmental conditions limits its reliability as a standalone marker. Variations in δ^{34}S arise from differences in sulfur sources (such as elemental sulfur commonly used in organic farming), soil sulfur cycling, and other environmental factors, which can cause δ^{34}S values to fluctuate independently of farming method. Therefore, δ^{34}S is most effective when combined with complementary markers like P to enhance the accuracy of agricultural authentication.

Misclassifications, such as C16, underline the challenges of relying solely on elemental and isotopic markers for differentiation. They highlight the need for additional analytical methods, such as FTIR, to improve classification accuracy and address borderline cases. No significant differences in δ^{15}N values were found between organic and conventional cinnamon, indicating that δ^{15}N alone is not a reliable marker for agricultural production methods across all food products. However, geographical variations in δ^{15}N were observed, particularly between Indonesian and Sri Lankan cinnamon, reinforcing the need to account for origin in interpreting isotopic data.

4. Conclusions

This study highlights the effectiveness of an integrated analytical strategy—combining isotopic, elemental, spectroscopic, and antioxidant profiling with advanced chemometric modeling—for the authentication and compositional assessment of paprika and cinnamon. Significant differences were found between the two spices, with cinnamon, especially Sri Lankan cassia, showing much higher antioxidant activity (AA) and total phenolic content (TPC) than paprika. The strong positive correlation between AA and TPC in cinnamon highlights the impact of species on bioactive compounds. While cinnamon's chemical composition varied notably by geographical origin, paprika exhibited relatively little variation across origins.

Stable isotope and elemental profiling enabled the robust classification of geographical origin and agricultural production methods for paprika and cinnamon. For paprika, origin prediction achieved 90% accuracy using key markers such as δ^{34}S, Mg, Sr, Cs, Rb, V, δ^{13}C, Fe, Al, P, S, and Ba, effectively differentiating samples from Hungary, Serbia, and Spain. Organic paprika samples were characterized by elevated δ^{15}N, δ^{34}S, and Zn, while conventional samples showed significantly higher concentrations of Na, Al, Cr, Pb, and V, consistent with synthetic fertilizers and agrochemicals.

In cinnamon, classification by origin reached 89% accuracy, separating samples from Sri Lanka, Madagascar, and Indonesia using Rb, Cu, Ca, Se, δ^{13}C, Na, δ^{34}S, Cs, Mn, δ^{15}N, Al, V, Cd, and Fe as key markers. The differentiation of agricultural production methods yielded 95% accuracy, identifying δ^{34}S, Ba, and P as the most influential markers. While organic cinnamon typically exhibited higher Ba and δ^{34}S values, the interpretation of δ^{34}S was complicated by its strong dependence on geographical origin. Furthermore, δ^{15}N did not reliably distinguish between organic and conventional cinnamon, highlighting the need to interpret isotopic data in the context of both origin and farming practices.

Lower classification and overlapping of the statistical evaluation according to origin were further supported by FTIR analysis, indicating possible adulteration. FTIR added complementary insights, effectively distinguishing Ceylon from cassia cinnamon and revealing potential adulteration in paprika. Further, in cinnamon, distinct bands at 780 and 750 cm^{-1} enabled species identification and reflected differences in farming practices, corroborating the AA and TPC results.

The distinct profiles observed across species, geographical origins, and production methods demonstrate the necessity of integrated multi-marker approaches for reliable spice authentication. However, ensuring adequate numerical representation of each product type, along with confirming their provenance and composition, is essential to strengthen the model's reliability and applicability across real-world scenarios. This was not fully achievable in the present study and will therefore be prioritized in future work by expanding the sample size and applying rigorous selection criteria to better capture the variability of the products under investigation.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/foods14132323/s1, Table S1: Inventory of the paprika powder samples with sample designation, description, geographical origin, and agricultural method of production; Table S2: Inventory of the cinnamon samples with sample designation, description, geographical origin, and agricultural method of production; Table S3: Antioxidant Activity (AA; mg TE/g LV) and Total Phenolic Compounds (TPC; mg GA/g LV) in paprika samples extracted with 70% ethanol. Values are presented as the mean ± standard deviation; Table S4: Antioxidant Activity (AA; mg TE/g LV) and Total Phenolic Compounds (TPC; mg GA/g LV) in cinnamon samples extracted with 70% ethanol. Values are presented as the mean ± standard deviation; Table S5: Wavenumbers (cm^{-1}) of identified peaks in IR spectra of paprika samples; Table S6: Correlation coefficients between IR spectra of paprika samples; Table S7: Wavenumbers (cm^{-1}) of identified peaks in IR spectra of cinnamon samples; Table S8: Correlation coefficients between IR spectra of cinnamon samples; Figure S1: Box plots of elemental concentrations (Fe, Zn, As, Rb, Ba, Pb, Cs) in paprika samples by agricultural method of production where statistical differences were observed.

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Abbreviations

The following abbreviations are used in this manuscript:

AA	Antioxidant Activity
DPPH	2,2-Diphenyl-1-picrylhydrazyl
TPC	Total Phenolic Content
FTIR	Fourier-Transform Infrared Spectroscopy
IRMS	Isotope Ratio Mass Spectrometry
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
PCA	Principal Component Analysis
OPLS-DA	Orthogonal Partial Least Squares Discriminant Analysis
DA	Discriminant Analysis
ED-XRF	Energy-Dispersive X-ray Fluorescence
EU	European Union
SIMCA	Soft Independent Modeling by Class Analogy

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3.5 Scientific Conference Paper: "Implementing an IoT System for Sea to Fork Transparency and Consumer Engagement"

This chapter presents the conference paper entitled "Implementing an IoT System for Sea to Fork Transparency and Consumer Engagement" by Tomo Popović, Dejan Drajić, Nives Ogrinc, Cathrine Terro, and Vladimir Urošević. The paper was published in the proceedings of the 28th International Conference on Information Technology (IT2024), Žabljak, Montenegro, February 21–24, 2024.

This paper describes an approach to implementing an IoT system for fish farmers, with open integration and support to the end-to-end sea-to-fork digital platform for transparency, seafood traceability, and consumer engagement. The system is implemented as a Cloud SaaS solution providing digital tools for easier fish farm management, easily accessible via the Internet using desktop/laptop or mobile devices.

The paper discusses the overall sea-to-fork platform and then focuses on IoT sensors and system requirements. A discussion of the initial evaluation of the pilot system, implemented in accordance with the requirements, is provided at the end. The solution is open for adding different IoT sensors that can be installed in other stages of the seafood value chain, such as transport, packing/processing facilities, and retail. The system is also open to using QR codes to identify items at different stages of the chain, e.g., trade units or final products, to enable interaction with various stakeholders and to motivate seafood consumers to engage with the overall platform.

28th International Conference on Information Technology (IT)
Žabljak, 21 – 24 February 2024

Implementing an IoT System for Sea to Fork Transparency and Consumer Engagement

Tomo Popović, *Senior Member, IEEE,* Dejan Drajić, *Senior Member, IEEE,* Nives Ogrinc, Cathrine Terro, Vladimir Urošević

***Abstract*—This paper describes an approach to implementing an IoT system for fish farmers, with open integration and support to the end-to-end sea to fork digital platform for transparency, seafood traceability and consumer engagement. The system is implemented as a Cloud SaaS solution providing digital tools for easier fish farm management, easily accessible via Internet using desktop/laptop or mobile devices. The paper discusses the overall sea to fork platform, and then focuses on the specifics related to the IoT sensors and systems requirements. A discussion on initial evaluation of the pilot system implemented according to the requirements is given at the end. The solution is open for adding different IoT sensors that can be installed in the other stages of the seafood value chain, such as transport, packing/processing facilities, and retail. The system is open to utilization of QR codes to uniquely identify items in different phases of the chain, e.g., trade units or final products, to allow interaction with different stakeholders and motivate seafood consumers to engage with the overall platform.**

***Index terms*— aquaculture, cloud, food transparency, fish farming, internet of things, seafood value chain.**

I. Introduction

The rise in urbanization and the expansion of retail infrastructure have brought about substantial changes in dietary patterns, causing disruptions to well-established ecological, agricultural, cultural, and economic practices [1]. Research indicates that nutritional intake has approached an optimal level in certain European countries; for instance, 57.8% of participants have reduced their consumption of unhealthy fast food. Nevertheless, the consumption of seafood and its exports have declined, attributed to heightened fish prices, diminished consumer demand, challenges in market access, and logistical issues arising from transportation and border restrictions. Addressing the challenges outlined in FOOD 2030 [2], particularly in the face of anticipated global food demand growth of approximately 60% by 2050, as projected by the FAO, necessitates innovative measures that can establish sustainable, resilient, responsible, diverse, competitive, and inclusive food systems. This transformation should occur within the framework of a circular bioeconomy, also known as the blue economy, aiming to provide a healthy diet while actively engaging European communities.

Digital transformation of agriculture is reflected in penetration of modern information technology into different areas of agriculture and mariculture, resulting in concepts of smart fish farming based on IoT sensors, edge computing Cloud platforms, mobile apps, 5G, and AI [3]. While the need for digitalization is recognized across the food value chains, including seafood-based food systems, there are still several challenges for increased uptake, some of which include interoperability issues, maturity of digital tools, lack of trust among the stakeholders, readiness to adopt new solutions, digital divide, etc. FishEUTrust project has an ambition to conceptualize a data-driven platform that will enable and motivate all stakeholders in the seafood value chain to participate and exchange their information with others, along with implementation of novel safety and quality sensors and control systems. This approach allows the participants to, while using various digital tools, expand their insight and increase their trust in the information across the value chain [4]. The overall platform will rely on a value-chain-wide data exchange based on standardized and open data formats and protocols, and the use of Blockchain and/or product passport technology [5-6].

IoT based systems for smart agriculture, and specifically for monitoring fish farms and water quality have been around for some time [7-10]. In this paper, we focus on implementing an IoT system for fish farms that will be open for integration into an overall digital platform for sea to fork transparency and consumer engagement. As such, this IoT system pilot will serve as a basis to create a testbed in Montenegro with a possibility to eventually upgrade to a co-creation living lab.

The paper is organized as follows. Section II gives the project background and elaborates on the motivation for the research. Section III focuses on the system requirements and considerations for IoT sensors system solution. Section IV provides details on the implementation and discussion.

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II. Project Background

Currently, seafood constitutes 17% of global animal protein production (179 million tonnes, 88% for human consumption), with aquaculture contributing 52% to the total production and serving as the primary source of fish for human consumption [11]. While fish farming is widespread in Europe, research and development efforts have predominantly focused on technical aspects of production. The current fragmented landscape across various disciplines and sectors lacks a more integrative perspective.

Both scientists and policymakers agree that there should be a shift towards emphasizing the environmental and social aspects of the industry. This involves promoting increased transparency and traceability. Despite the high quality of seafood products, limitations in product traceability and microbiological safety methods exist and must be significantly improved to enhance consumer confidence and, consequently, uptake. Consumers often encounter fish and shellfish with unclear origin details and minimal information about various aspects such as fishing methods, feed, welfare standards, processing, and transport. This lack of information exacerbates existing trust issues, particularly concerning farmed seafood. Furthermore, EU consumers express a strong interest in obtaining more information about the fish they purchase, including details such as the date of catch or harvest and the conditions of growth [4]. The increasing demand for environmental information, and to a somewhat lesser extent, ethical and social information, underscores the necessity of adopting a Farm-to-Fork strategy [12]. This approach empowers consumers to make informed choices and opt for sustainable products.

The core innovation of FishEUTrust project lies in the integration of diverse stakeholders, actors, and consumer sectors within a unified platform [4]. This platform serves as a nexus connecting technology providers with supply chain stakeholders, fostering direct engagement with socioeconomic analysis. The aim is to create optimized business models and collaborate with regulatory/policy actors in the co-development of tools, systems, and protocols across the seafood value chain. The overarching goal is to enhance consumer awareness, engagement, and confidence in the seafood industry. By facilitating collaboration and information exchange among various players, the project seeks to create a cohesive and effective approach to address challenges and drive positive transformations in the seafood sector as illustrated in the schematic shown in Fig. 1.

Numerous technologies, systems, tools, and protocols required to address the challenges discussed earlier already exist. Many of these have been successfully demonstrated in various sectors, often unrelated to the issues at hand. However, the problem lies in the fact that these solutions exist in isolated silos of excellence, lacking integration into a cohesive platform. These existing technologies are disparate and fragmented, hindering their collective impact. The absence of an integrated or cohesive platform results in missed opportunities for potential solutions. The challenges are compounded by the multidisciplinary nature of the requirements and the involvement of a large number of stakeholders from diverse sectors. These stakeholders cater to a wide range of consumer demands and requirements across the complex value chain, making it challenging to streamline efforts and find comprehensive solutions [4].

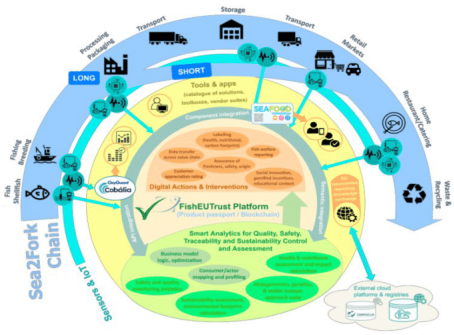


Figure 1. Schematic overview of the FishEUTrust concept [4]

The scope is to define the framework for the overall FishEUTrust platform and integration of all data that everyone has to offer. Information is gathered trough standardized and common information model, standardized for the project and in line with existing standards, which is included in the overall project platform. The platform collects information and data (inputs/outputs) coming from every type of stakeholder or partner. Common information model is standardized for everybody participating in this platform. The project combines cutting-edge technology with practicality to address challenges in the seafood industry efficiently. Existing and commercially available low-cost sensors are integrated with the FishEUTrust platform (Section III). On the other hand, the project partners will embark on the development of innovative sensors with an ambition to detect pathogens, antibiotics and toxins. For these sensors the platform provides APIs for smooth integration.

III. Sensors & IoT System Requirements

In this paper we are describing the sensors and IoT layer of the overall FishEUTrust platform (Fig. 2). The idea is to provide an IoT based system for managing sensor data across the fish value chain. The platform is to be open for information exchange with the various digital tools and applications that may be employed by different stakeholders throughout the chain. For transparency and traceability purposes the IoT sensor data will be made available for the FishEUTrust platform that may utilize product passport and/or distributed ledger technology (blockchain). Having in mind the implementation of co-creation living labs for the project, the proposed IoT system is primarily focused on smart fish farms, while allowing interactions with other stakeholders across the supply chain.



Figure 2. Context view of the proposed IoT system

A. Related Work

As mentioned, authors in [7-10] and [14] proposed the use of IoT sensors and systems for water quality and fish pond monitoring. In [14], the authors conducted a review of the utilization of intelligent equipment in fisheries, along with the integration of IoT, edge computing, 5G, and artificial intelligence algorithms in contemporary aquaculture. The paper also examines current challenges and potential future development prospects in this field. In [15], authors outlined various business processes within the fishery and aquaculture value chain, identifying key stakeholders participating in activities such as sardines canning and cod fish salting industries. Paper [16] focuses on designing and implementing an IoT-based system for the real-time monitoring, control, and management of fish farming. The system monitors crucial water parameters, including pH value, water temperature (T), dissolved oxygen (DO), salinity (S), ammonia (NH3), and transparency (Tr). Data transmission occurs through a WiFi network, albeit with limited availability, to the cloud. The device is currently powered by electricity, and the integration of solar power generation is identified as a potential aspect for future work. In review article [17], authors discussed the traceability systems of sustainable and safe fisheries supply chain management using the RFID technology. The authors in [18] proposed a smart contract to be used on the Ethereum blockchain for the implementation of the traceability platform. For the end consumer or authorities to conveniently access the information stored on the blockchain, it is essential to develop a tool that presents this information in a user-friendly and easily navigable interface. The paper [19] introduces a managerial framework that serves as a foundation for the development of a fish traceability system. It outlines dimensions that fishery industry stakeholders, programmers, and government entities can take into account as they contemplate the construction of a traceability system. Feasibility of Blockchain based traceability for fish food systems was discussed in [5,6], as well. Blockchain is recognized as a technology enabler for agri-food supply chain traceability [20]. For us, it is interesting to consider implementation of IoT system that is open for integration into the overall end-to-end seat to fork digital platform.

B. Sensors Requirements and Selection

The initial selection of IoT sensors that need to be supported are identified in Table I. The majority of sensors are to be used at fish farms to monitor water quality parameters. In addition, it is anticipated to monitor transports using GPS location and sensors within trucks. QR codes are to be used to initiate the interaction between users/ stakeholders outside the fish farm, to provide them with the information about the product/package and to collect additional feedback from them. QR codes are particularly convenient for the use with mobile devices where the user can open the web-based mobile interface by scanning the QR code on the product label or accompanying documentation. Finally, the system needs to be open for further expanding and new sensors, especially ones for pathogens, antibiotics and toxins that are expected to be available by the end of the FishEUTrust project.

TABLE I
IOT SENSORS TO BE SUPPORTED

Sensor	***Range***	***Periodiciy***
Dissolved Oxygen (DO)	0~20 mg/L	15m
Electrical Conductivity (EC)	0~500us/cm, 0 ~ 5000us/cm, 0 ~ 10000us/cm, 0 ~ 200000us/cm	15m
pH value	0~14 pH	15m
Oxidation Reduction Potential (ORP)	0 ~ ±1500 mV	15m
Water Temperature (T)	0 ~ 100 ºC	15m
GPS	coordinates	15m
Camera	image	1d, per request
QR codes	n/a	per request
New sensors, if needed	data/status	per request

From the fish farming perspective, the following selected sensors have an important role in the water quality monitoring:

- The DO sensor is important for measuring the amount of oxygen in the water, which is important for the fauna and flora in the water. In water quality controls such as in aquaculture and ponds, dissolved oxygen levels must be high. If the concentration of dissolved oxygen falls below the permissible limit, the fish will suffocate.
- Conductivity gives an idea of the amount of minerals and salts dissolved in the water, which can also be related to the amount of chemicals dissolved in the water.
- The pH value is a measure of the acidity or alkalinity of water. If this value is out of the desired range, aquatic life is endangered, e.g., below pH=6 trouts are endangered.
- ORP is one of the indicators in determining the amount of life in water. The higher the ORP potential, the more potential the water has to oxidize and thus destroy organisms and to destroy them faster. It can serve as an indication of pollution, especially for industrial wastewater. ORP reflects the antimicrobial potential of water.
- Water temperature is a very important parameter, especially from the aspect of indirect influence on the living world in aquatic ecosystems. The solubility of oxygen (diss. O_2) and carbon dioxide (CO_2) gases directly depends on the temperature.

The IoT sensor nodes should be equipped with a battery to endure through temporary power outages (Fig. 3). The device can be attached to a standard AC power supply or utilize an optional solar panel (where AC power is unavailable). In addition, the enclosure should be fit for outdoor installation and with proper dust and water resistance (like IP67). The default temporal measurement resolution is 15 minutes (configurable) and data is transferred via mobile network (4G) to the Cloud.



Figure 3. Solar powered IoT sensor node with 4G and IP67 enclosure

C. *Software Requirements*

The goal of the development is to implement an IoT based smart fish farm management system that will fit into the overall project context. The following summarizes the main software requirements:

- Implement the system as a Cloud-based Software as a Service (SaaS) solution easily accessible via Internet using desktop/laptop and/or mobile devices with different screen sizes and resolution,
- Integration of data from the following sensors: water temperature, pH levels, dissolved oxygen, Oxidation-Reduction Potential, liquid EC and salinity sensors,
- User and user role management, management of sensor nodes and sensor setup, and fish farm configuration;
- Implementation of the APIs for the selected set of IoT sensors and data integration using standardized protocols and data formats;
- Sensor data visualization (trend charts), and export of measurements data into CSV and XLSX formats for offline analysis;
- Plan for integration of data from truck monitoring: GPS location and on-board environmental sensors;
- Support for QR code generation that uniquely identifies corresponding trade units and/or final product;
- Open for adding different reports customized for different types of stakeholders across the value chain; access to these reports can be initiated through QR code scanning;
- Open for adding new sensors and integration with new analytic tools expected to be developed by the end of the project (pathogens, antibiotics, toxins).

IV. Implementation and Discussion

The software architecture is depicted on Fig. 4. The IoT system is implemented as a Cloud based SaaS application that follows a three-layer system architecture.

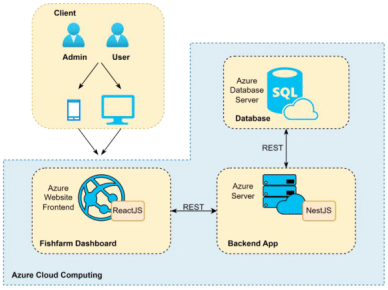


Figure 4. IoT System architecture: 3-layer Cloud based SaaS

The presentation (view) layer is implemented using React.JS as web-based responsive user interface that works both on desktop and mobile devices with different screen sizes [21]. Fish farmers can easily monitor the water quality and situation at the farm. In the near future, there will be provisions to manage the feeding process as well. After logging on to the platform, the platform home page will be presented, where locations of the available IoT sensor nodes could be seen (Fig. 5).

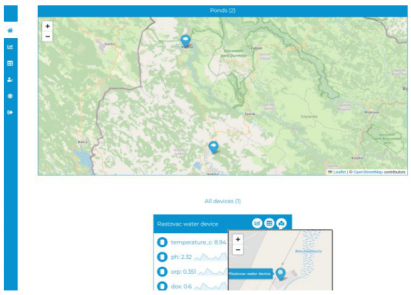


Figure 5. Viewing sensor data using web-based desktop access

By clicking on the specific node the following sensor data are available: trend charts with zoom in/out and tabular view for the selected date/time range for each selected sensor (Fig. 6). Fish farm IoT system could be also accessed by using a smart phone (Fig. 7).

On the business layer (back-end), we have the module that is responsible for exchanging the IoT sensor data from various types of sensors using standardized APIs. Currently, most of the sensors are related to environment monitoring, and since we are using Azure IoT hub [22], then the MQTT and HTTPS protocols are supported, and in our case, we are using HTTPS POST and JSON file format. With respect to the whole value chain, the current version supports the fish farm and transport monitoring, as well as the interaction with consumers using QR code in the GS1 standard [23]. As we progress with the detailed specification of business processes across the value chain, the platform is expected to "grow" and support the management of additional sensors and necessary IoT data. The back end was implemented in NestJS and hosted on Azure Cloud [24].



Figure 6. Viewing sensor data: a) trend charts with zoom in/out (top); and b) tabular view for the selected date/time range (bottom)

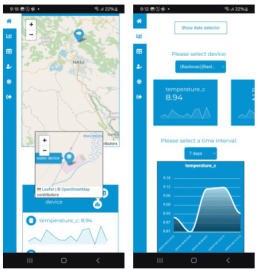


Figure 7. Accessing the fish farm IoT system using a smart phone

Current data layer is focused on managing data and reports from the farmers' perspective, while allowing the creation of product information summary for the consumers. We anticipate creation of additional reports customized for different stakeholders across the value chain (logistics, processing, packing, retail, etc). The overall project platform will also allow for different gamification strategies and interactions with consumers and other stakeholders, in order to raise awareness with respect to seafood consumption benefits, food safety and authenticity [25].

In the future work, we intend to implement cold storage monitoring in trucks for fish transport in order to monitor transport conditions (air temperature and relative humidity) and truck routes by using a GPS module. We anticipate that each instance of the end product will be assigned a unique ID number (GS1) and that can be traced back to the source using FishEUTrust data platform. Each instance of the product or trade unit could be equipped with a smart label, using QR code, NFC, RFID or similar technology that would allow for easier interaction between physical instance of the product and its digital counterpart in the data platform. Further, the developed system represents a foundation of the traceability system established within FoodTraNet project [26], and can be easily applied to other food supply chains.

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Chapter 4

Conclusions

This dissertation tackled the critical issue of food fraud by proposing and validating a comprehensive, integrated framework built around three traceability systems: (1) Physical Traceability, (2) Rapid Traceability, and (3) Digital Traceability. Focusing on high-value commodities like saffron, paprika, and cinnamon, which are particularly vulnerable to fraudulent practices such as mislabeling and adulteration, this research demonstrated how combining stable isotope and elemental analysis, rapid spectroscopic methods, and IoT-based monitoring technologies can significantly elevate the reliability, transparency, and scalability of food authenticity verification across global supply chains.

The results confirm the power of this integrated approach, validating the thesis's core:

H1: Stable isotope and elemental analysis can successfully differentiate between conventional and organic production, as well as verify the geographical origin of saffron, paprika, and cinnamon.

The work provides strong evidence in support of the H1. In the saffron study, stable isotope ratios (δ^{2}H, δ^{13}C, δ^{15}N, δ^{18}O, δ^{34}S) and multi-elemental analysis (e.g., K, Ca, Mn, Sr, Zn) achieved 94% classification accuracy in identifying saffron's geographical origin. The use of these isotopic and elemental markers demonstrated their ability to distinguish saffron from different regions, including Morocco, Greece, Slovenia, Spain, Iran, and the United Kingdom, with high sensitivity and specificity. Key isotopic and elemental markers (δ^{2}H, δ^{15}N, K, Ca, δ^{13}C, Sr, Mn, Co, and Zn), along with elemental ratios (Rb/Sr, K/Rb, Ca/Sr) and/or δ^{18}O and δ^{34}S, provided strong discriminatory power, enabling precise differentiation, particularly for Moroccan saffron.

Similarly, for paprika, origin prediction achieved 90% accuracy using key markers such as δ^{34}S, Mg, Sr, Cs, Rb, V, δ^{13}C, Fe, Al, P, S, and Ba, effectively differentiating samples from Hungary, Serbia, and Spain. Organic paprika samples were characterized by elevated δ^{15}N, δ^{34}S, and Zn, whereas conventional samples showed significantly higher concentrations of Na, Al, Cr, Pb, and V, consistent with synthetic fertilizers and agrochemicals.

In cinnamon, classification by origin reached 89% accuracy, separating samples from Sri Lanka, Madagascar, and Indonesia using Rb, Cu, Ca, Se, δ^{13}C, Na, δ^{34}S, Cs, Mn, δ^{15}N, Al, V, Cd, and Fe as key markers. The differentiation of agricultural production methods yielded 95% accuracy, identifying δ^{34}S, Ba, and P as the most influential markers.

H2: The developed method could be used to assess the authenticity of commercial spices on the market, with the expectation that more than 80% of spices are correctly labelled.

The DD-SIMCA classification model effectively distinguished saffron from different geographical regions, achieving high specificity (93.6% to 99.0%) and sensitivity (94% to 99%) across samples from Italy, Morocco, and Iran. This model's ability to accurately classify saffron using isotopic and elemental markers proves that our method can reliably verify the authenticity of saffron on the market, aligning with the hypothesis that authenticity assessments can achieve high accuracy.

Despite the overall success, the model also identified mislabeling, with approximately 15% of the commercial samples misclassified. This misclassification includes saffron samples labeled as originating from regions like Spain and Greece but classified as non-authentic, confirming that our method can detect incorrect labeling. This result underscores that a significant portion of market spices are indeed misrepresented, validating the expectation that over 80% of spices are correctly labeled when using our advanced model.

In addition, FTIR added complementary insights, effectively distinguishing Ceylon from cassia cinnamon and revealing potential adulteration in paprika.

The method not only confirms the authenticity of labeled saffron but also highlights a concerning level of mislabeling, validating the hypothesis that the developed model can assess authenticity in commercial spices and has the potential to identify a significant proportion of mislabeled products.

H3: The integration of AI-driven verification methods improves the classification accuracy of food authenticity compared to traditional statistical modeling.

The integration of AI-driven verification methods enhanced the classification accuracy of saffron origin compared to traditional statistical modeling approaches. The OPLS-DA approach was limited by sparse data, and Spain was excluded due to the small sample size. In contrast, the AI model included Spain, achieving 100% classification accuracy for these samples despite class imbalance. The AI model delivered superior overall performance, achieving 93.07% accuracy and 90.11% balanced accuracy using combined stable-isotope and elemental analysis, with LightGBM emerging as the optimal algorithm.

SHAP explainability analysis also revealed that elemental concentrations, particularly nickel and cobalt, provide the primary discriminatory information, while stable isotopes

contribute negligibly to classification performance. This enhanced accuracy and ability to handle imbalanced datasets confirm AI's advantages for real-world verification scenarios.

H4: IoT-enabled real-time monitoring of fish farms (DO, ORP, pH, EC, water temperature) enhances aquaculture management and ensures seafood quality.

The IoT pilot implemented under the FishEUTrust initiative validated the hypothesis that digital traceability systems strengthen transparency and operational control in aquaculture. The developed Cloud-based SaaS solution integrated multiple water-quality sensors and real-time data visualization, enabling continuous monitoring of parameters critical to fish welfare and product quality. The system supported early anomaly detection, remote management, and full traceability from farm to consumer through QR code and blockchain-compatible modules. The results confirmed that IoT technologies complement physical verification methods, bridging the gap between analytical authenticity testing and dynamic supply chain transparency.

While the results of this dissertation demonstrate the validity and potential of the proposed integrated framework, its evaluation remains at a proof-of-concept stage. The physical traceability component was assessed using authenticated saffron samples from selected geographic origins and a limited set of commercially sourced paprika and cinnamon samples from the Slovenian market. Although these datasets provided valuable insight into real-world mislabeling and fraud patterns, they do not capture the broader diversity of spice production systems, geographic origins, and adulteration practices present in international supply chains. Expanding the dataset to include additional fraud-prone spices, such as turmeric, black pepper, cumin, and oregano, as well as authenticated samples from a wider range of countries and production systems, would be necessary to better assess the generalizability of the proposed framework.

Scaling the framework to national or international regulatory use would require expanded reference databases for other commodities, harmonized inter-laboratory protocols, including proficiency testing and round-robin exercises, and coordinated data-sharing agreements across institutions and borders. A phased scaling approach, beginning with national pilot programs involving certified laboratories and regulatory authorities, followed by cross-border harmonization through established structures such as the EU Food Fraud Network, would provide a realistic pathway toward broader implementation.

The AI-driven model, while achieving high classification accuracy and demonstrating potential for reducing analytical costs through SHAP-guided elemental feature selection, was trained on a geographically limited dataset. Its generalizability is further constrained by the small saffron sample sizes for certain origins, particularly for Morocco and Spain. To mitigate overfitting, we implemented a rigorous evaluation strategy comprising stratified five-fold cross-validation, SMOTE applied only to the training folds, feature scaling, and Bayesian hyperparameter optimization—ensuring the model was always tested

on real, unseen data with no information leakage across folds. Nevertheless, since all samples originate from the same curated database, external validation using independent saffron samples from additional regions, harvest years, and potentially other commodities remains essential to fully confirm the model's robustness and generalizability. Similarly, the IoT pilot, implemented at a single fish farm in Montenegro under controlled conditions, would face logistical, technical, and financial challenges if expanded to diverse farm sizes, infrastructure capacities, and regulatory environments.

Economic feasibility remains a critical consideration. The capital investment required for IRMS and ICP-MS instrumentation, ongoing database curation, AI model retraining, and deployment and maintenance of IoT sensor networks may pose significant barriers, particularly for smaller producers and regulatory bodies in resource-limited regions. Stakeholder adoption across the supply chain, including producers, laboratories, regulators, retailers, and consumers, cannot be assumed and will depend on demonstrated added value. This should be assessed through metrics such as cost-per-sample reductions relative to current enforcement practices, improvements in fraud detection rates, inter-laboratory reproducibility across different instrument configurations, and consumer engagement with QR-enabled traceability information.

These limitations do not diminish the scientific value of the work, but they do indicate that further validation, expansion, and stakeholder testing are needed before the framework can be considered fully transferable to broader real-world settings. Such efforts are essential to translate the scientific promise of this integrated framework into sustained, practical impact across diverse food supply chains.

Future Perspectives

Building on this research, several promising future directions should be explored to advance the field of food authenticity and traceability. The future of this field will involve integrated, interdisciplinary approaches that combine analytical chemistry, data science, digital technologies, and policy frameworks.

For the IsoFoodTrack platform, future work should prioritize expanding both geographic and commodity coverage through international collaborations and open-access data-sharing initiatives, so that laboratories and research infrastructures across diverse agro-climatic regions contribute reference datasets spanning major food groups. In parallel, connecting IsoFoodTrack with global repositories such as IsoBank and with FAIR (Findable, Accessible, Interoperable and Reusable) data networks would allow harmonized metadata standards, shared ontologies and common exchange formats, thereby strengthening cross-validation of datasets, improving methodological consistency and supporting transparent data flows between academic, regulatory and industrial stakeholders. Implementing machine-readable datasets and open APIs would then enable seamless access for laboratories, customs authorities and certification bodies, facilitating near real-time authenticity checks and automated decision support; in this way,

IsoFoodTrack could evolve into a cornerstone of digital food-provenance infrastructures that underpin evidence-based policy, consumer protection and coordinated action against food fraud.

The demonstrated success of machine learning models underscores the need to develop explainable AI (XAI) systems capable of transparent decision-making. Future research should explore deep learning architectures, transfer learning, and automated retraining of models using continuously updated data from IsoFoodTrack. Embedding XAI into web-based analytical pipelines will make authenticity verification both interpretable and traceable, enabling regulators and producers to understand why a product was classified as authentic or fraudulent. The ultimate goal is to evolve from static classification toward self-improving AI systems that dynamically adapt to new fraud patterns and analytical updates.

At the analytical level, future research should further integrate compound-specific isotope analysis (CSIA) and metabolomic profiling with existing bulk isotope and elemental approaches. Targeting individual amino acids is particularly powerful because their carbon and nitrogen isotope signatures retain information on nitrogen sources and trophic or metabolic pathways, allowing finer discrimination between farming practices (for example, synthetic versus organic fertilization) and between closely related geographical origins that might overlap at the bulk level (Liu et al., 2023). Recent CSIA applications to foods such as wine, honey and walnuts have shown that $\delta^{13}C$ and $\delta^{15}N$ patterns of selected amino acids can clearly separate regional origins and organic production schemes that are indistinguishable at the bulk level (Liu et al., 2023; Athaillah et al., 2023). Compound-specific analysis of lipids and phenolic compounds has increased sensitivity for detecting low-level adulteration in high-value edible oils and other products (Liu et al., 2023). Extending CSIA to these additional molecular classes and combining the resulting data with metabolomic or spectroscopic fingerprints will therefore generate high-dimensional ‘chemical passports’ that capture subtle environmental, climatic and processing effects and improve the detection of complex blending strategies (Liu et al., 2023).

From a policy perspective, EU food traceability and authenticity will depend on integrating AI, machine learning, and blockchain into existing systems and safety standards. Policymakers should prioritize a holistic, risk-based framework that centralizes interoperable data for early fraud detection and transparency. This requires developing interoperable digital systems, public-private partnerships, and independent data trusts. Strengthening frameworks like the Farm to Fork Strategy (European Commission, 2020) with AI solutions and real-time data exchange between authorities and businesses is essential (European Commission JRC, 2024; European Parliament & Council, 2024). Finally, incentivizing industry adoption of digital tools while engaging consumers to demand transparency will create a more secure, resilient, and fraud-resistant food system.

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Conference Paper

Popović, T., Drajić, D., Ogrinc, N., Terro, C., & Urošević, V. (2024, February). Implementing an IoT system for sea to fork transparency and consumer engagement. In 2024, the 28th International Conference on Information Technology (IT) (pp. 1–5). IEEE. https://doi.org/10.1109/IT61232.2024.10475712

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Ogrinc, N., Potočnik, D., Terro, C., Strojnijk, L., Jagodic Hudobivnik, M., Mazej, D., Simčič, A., Vehar, A., Modic, R., Simčič, A., Ogrinc, M., Drole, J., Eftimov, T., Koroušić-Seljak, B., Bontempo, L., & Roncone, A. (2024). Report on IRMS and ICP-MS characterization of raw materials and validation of an IT system for geographical traceability of PROMEDLIFE raw materials: Deliverable 3.3. Ljubljana: Institut Jožef Stefan. [COBISS.SI-ID 203349763]

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Biography

Cathrine Terro began her academic journey at the American University of Beirut, Lebanon, earning a Bachelor of Science in Nutrition and Dietetics in 2017. She then completed a Master's degree in Food Security and Human Development at Università degli Studi Roma Tre in Italy in 2020. In 2021, she enrolled in the Ecotechnologies doctoral program at the Jožef Stefan International Postgraduate School (IPS) in Ljubljana, Slovenia, under the supervision of Prof. Dr. Nives Ogrinc, following her award of a prestigious Marie Skłodowska-Curie scholarship with the FoodTraNet European PhD Training Network. As part of this program, she joined the Jožef Stefan Institute (IJS) as an Early-Stage Researcher (ESR) in the Department of Environmental Sciences and is now employed there as a research assistant. Her doctoral research focuses on developing advanced and novel strategies for food authenticity and geographical traceability, combining stable isotope and multi-elemental data with statistical modeling and explainable artificial intelligence (XAI). Case studies include saffron, paprika, and cinnamon. She has also played a significant role in enhancing IsoFoodTrack, a database dedicated to preventing food fraud and ensuring traceability.

Cathrine expanded her training through research exchanges as part of the FoodTraNet project at Fondazione Edmund Mach in Italy, where she conducted laboratory work on grain and vegetable authentication, and at Concast – Trentingrana, also in Italy, where she contributed to dairy industry communication and marketing projects. She further expanded her skills at Food Forensics Ltd in the UK, gaining expertise in Fourier transform infrared (FTIR) spectroscopy and collaborating with international teams on food authenticity research. She has presented her research at numerous international conferences, including the International Mass Spectrometry Conference in Melbourne, Australia, and Maastricht, Netherlands; the ISO-FOOD Symposium in Portorož, Slovenia; and the International Symposium on Food Safety and Control in Vienna, Austria. Alongside her research, Cathrine has demonstrated strong leadership in academic communication: serving for three consecutive years as Communications Team Leader for the Jožef Stefan International Postgraduate School Students' Conference (IPSSC), serving as Vice President of the Student Council for three years, and working as Communications Team Manager for the FoodTraNet network. She has also been active in outreach as part of the FoodTraNet team, delivering several workshops during the European Researchers' Night. Currently, Cathrine contributes to other EU-funded research projects, including PROMEDLIFE and FishEUTrust, while continuing to build her expertise at the Jožef Stefan Institute.