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REVIEW

Geographical origin authentication of fruits: A decadal review (2014–2024) of technological progress and outlook

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Highlights

- Multi-omics integration improves accuracy but faces key markers selection challenges.
- Machine learning should shift from high-accuracy prediction to interpretable inference for origin certification.
- Agricultural practice variation reduces model generalization and should be included as a variable.

Abstract

Food fraud is an increasingly prevalent deliberate act of deception for profit. Hence, it is highly necessary to develop robust analytical methods to assess the authenticity of foods. In recent years, the geographical origin authenticity of fruits has attracted considerable public concern. The geographical origin of fruit is generally determined based on specific indicators such as elements, stable isotopes, and metabolites. Many studies have demonstrated that mineral elements and stable isotope ratios are effective indicators for geographical origin authentication as they are directly related to the geographical environment. Other techniques, such as spectroscopy and chromatography, also exhibit promising potential for fruit origin discrimination and authenticity assessment. Omics technologies have emerged as a key approach for authenticating the geographical origin of fruit. The integration of instrumental analysis techniques with machine learning enables high-precision discrimination of fruit geographical origin, and the growing trend toward combining multiple analytical techniques further enhances identification accuracy. Commonly used methods for geographical origin authentication include linear techniques such as PCA, PLS-DA, and LDA. Machine learning algorithms, including SVM, RF, and ANN, have also been applied to identify fruit origin with high accuracy. Future developments in this field should prioritize the consideration of agricultural practices to ensure reliable and practical authentication.

Keywords: fruit, authenticity, geographical origin, multivariate data analysis

1. Introduction

Fruits are an important part of food for humans due to their abundant carbohydrates, acids, vitamins, fiber, essential elements, and secondary metabolites (polyphenols, carotenoids, and anthocyanins) (Ruiz Rodríguez *et al.* 2021). Numerous studies have demonstrated the physiological functions of bioactive substances in fruits, such as alleviating tissue aging (Buachan *et al.* 2014), preventing cardiovascular diseases (Zhao *et al.* 2017), inhibiting atherosclerosis (Thilakarathna and Rupasinghe 2012), exerting anticancer effects (Tang *et al.* 2011), exerting anti-inflammatory effects (Taleb *et al.* 2016), exerting antioxidant effects (Hassimotto *et al.* 2005), exerting antimicrobial effects (Suriyaprom *et al.* 2022), exerting antiviral effects (Qanash *et al.* 2022),

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inhibiting cholesterol synthesis (Niwa and Niwa 2011), and lowering blood pressure (Tsubota-Utsugi *et al.* 2010).

China has the largest number of indigenous fruit trees in the world (Nie *et al*. 2024). In 2023, China enacted an updated Agricultural Product Quality and Safety Law, which encourages the establishment of food traceability systems to ensure food quality and security. Traceability regulations of food products have been implemented in other countries as well, such as the United States, Europe, Japan, Australia, and New Zealand (Shuai *et al*. 2022a). With increasing attention to food quality and safety, the geographical origin of food has become a great concern for both consumers and producers around the world. Implementation of origin tracing systems not only allows producers in specific regions to use origin labels and pursue premium prices, but also protects the consumers from unfair pricing and inferior or even hazardous products (Francois *et al.* 2020).

The quality of fruits is determined by their unique flavor, appearance, color, and nutritional value as the basic functional characteristics (Klee 2010). It is important to verify the authenticity of fruit geographical origin since their quality and internal characteristics are highly dependent on the geographical region and agricultural practices (Shuai *et al.* 2022a). In some countries and regions, some special planting practices have a good reputation for producing high-quality fruits. To facilitate the regulation and certification of origin labels, the geographical origin of fruit is verified through the protected designation of origin (PDO) and protected geographical indications (PGI) (Qian and Dong 2023). Fruit geographical origin authentication can protect the interests of farmers, reassure consumers, and significantly reduce fraudulent acts. China is the largest fruit producing country in both cultivated area and production in the world, followed by India and Brazil (Fig. 1-A and B). Fruit production is increasing year by year, and the global fruit production reached 887.027 million tons in 2020 (Fig. 1-C).

The main biochemical components of fruit are of great importance for origin traceability and authentication (Ruiz Rodríguez *et al.* 2021). Multivariate data analysis and data mining techniques have been extensively used to identify the geographical origin of agricultural products (Fig. 2). In terms of fruits, elements, stable isotopes, and metabolites are generally the critical variables. It is worth noting that maturity (Hou *et al.* 2020), storage conditions, cultivation measures (Santoni *et al.* 2016), soil types, and natural environmental conditions (mainly including temperature, precipitation, altitude, and latitude) are important factors for the formation of unique geographical characteristics of fruits (Fig. 3) (Andreu-Coll *et al.* 2021). Section 2 and Section 3 of this review specifically focus on the progress in fruit geographical origin traceability from 2014 to 2024. These sections provide a comprehensive overview of recent applications of analytical approaches to the determination of the geographical origin of fruits, and analyze factors that influence stable isotopic, mineral, and metabolite levels. Section 4 highlights the current challenges in this field and presents the prospects. Finally, Section 5 provides a conclusion based on the review.

2. Bibliometric analysis of fruit geographical origin identification

This review includes publications on fruit geographical origin authentication published between 2014 and 2024, retrieved from PubMed, ScienceDirect, Web of Science, and Google Scholar. The search terms include “fruit”, “geographical origin”, “stable isotopes”, “mineral elements”, and “metabolites”. The source and number of journals related to the geographical origin of fruits are shown in Fig. 4-A.

2.1. Countries contributing to fruit geographical origin authentication research

From 2014 to 2024, there were 146 articles focusing on fruit geographical origin authentication (Fig. 4-E), and 68 articles dedicated to fruit origin identification were selected for discussion in this review (Appendix A). During the period of 2014–2024, China emerged as a leading contributor in the field of fruit geographical origin authentication (Guo *et al.* 2020). In this period, China was the top country in terms of publications with 65 articles, representing 44.5% of the total number of included articles, probably because China is both the largest producer and consumer of fruit in the world (Fig. 4-D). Italy, Spain, Greece, and Egypt also contributed some articles. Italy had 23 articles on fruit traceability and

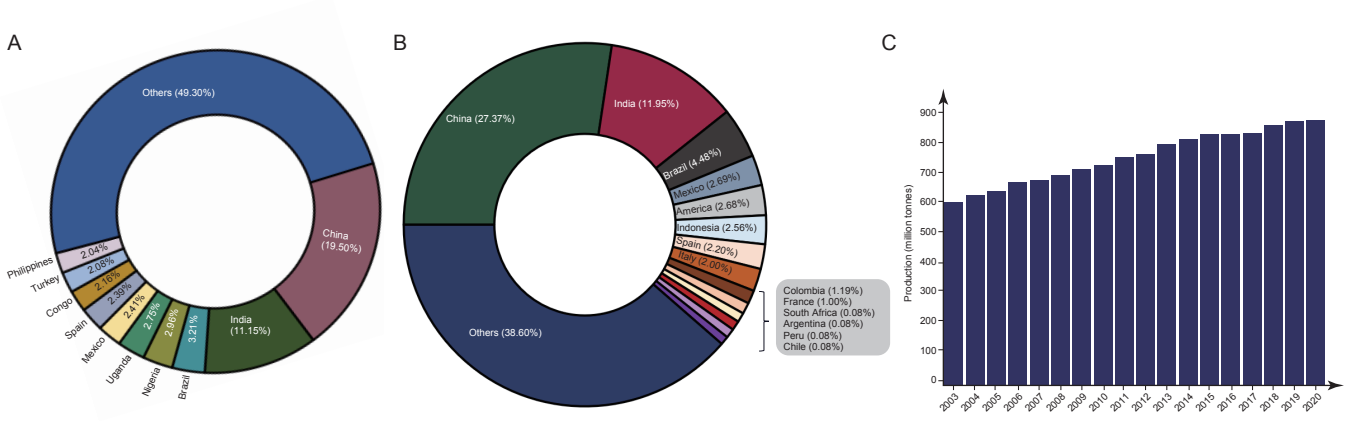


Fig. 1 Distribution of global fruit planting areas in 2020 (A), global fruit production in 2020 (B), and global fruit production from 2003 to 2020 (C).

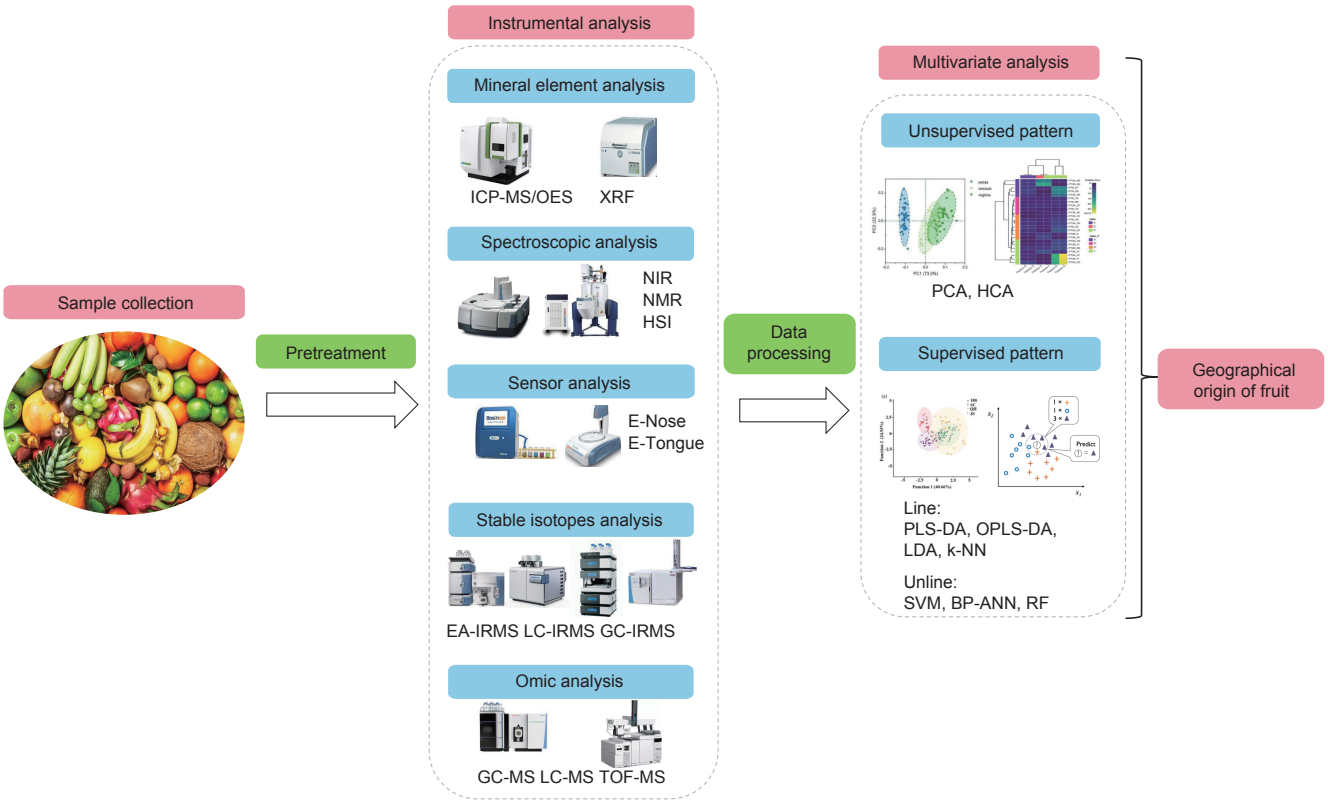


Fig. 2 Determination of the geographical origin of fruit using analytical techniques and multivariate analysis.

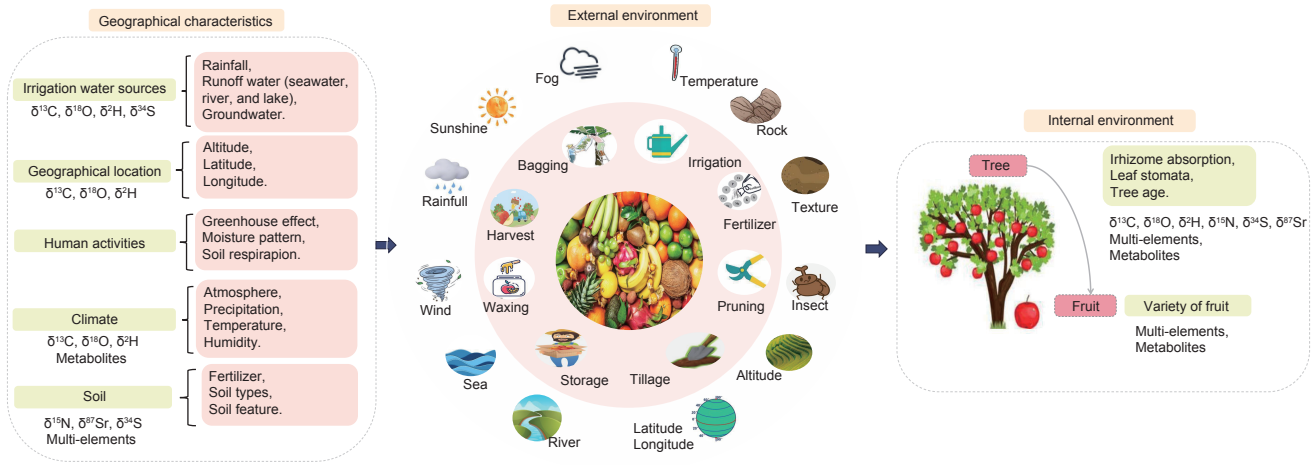


Fig. 3 Influence of various factors on stable isotope ratios, multi-elements, and metabolites in fruit.

authentication, while Spain, Greece, and Egypt had 12, 6, and 4 articles, respectively. Each of Portugal, Germany, the Netherlands, Slovenia, and Austria had three articles (Fig. 4-D). Studies have been conducted on the geographical origins of fruits in other countries, including Africa (Centonze *et al.* 2019), Brazil (Kornexl *et al.* 1996), Côte d'Ivoire (Muñoz-Redondo *et al.* 2021), England (García-Ruiz *et al.* 2007), Equatorial Guinea (Muñoz-Redondo *et al.* 2021), France (García-Ruiz *et al.* 2007), Italy (Giannetti *et al.* 2017), Mexico (Muñoz-Redondo *et al.* 2022), Peru (Muñoz-Redondo *et al.* 2021), Senegal (Muñoz-Redondo *et al.* 2021), Slovenia (Bat *et al.* 2012), Spain (Khanmohammadi *et al.* 2014), and Switzerland (García-Ruiz *et al.* 2007) (Appendix A). From 2014 to 2024, the investigated fruit species for geographical origin traceability included apple, avocado, banana, blueberry, cherry, durian, grape, hazelnut, kiwifruit, lemon, mango, mandarin, peach, pear, pomegranate, pomelo, quince, strawberry, wolfberry, and winter jujube (Fig. 4-B).

2.2. Journals publishing research on fruit geographical origin authentication

From 2014 to 2024, 61 journals published articles related

to fruit geographical origin identification. *Food Chemistry* published the most articles on fruit origin identification (17 articles), and its sub-journal *Food Chemistry: X* also published several related articles (7 articles). *Journal of Food Composition and Analysis*, *Food Control*, *Foods*, and *Journal of the Science of Food and Agriculture* published 10, 12, 11, and 8 articles respectively. A small number of articles on fruit origin identification have been published in *LWT-Food Science and Technology*, *Microchemical Journal*, *Molecules*, *Plants (Basel)*, *Food Research International*, and *Food Analytical Methods*. The remaining 49 journals published a total of only 57 articles on fruit geographical origin authentication (Fig. 4-A).

3. Techniques for fruit geographical origin authentication

3.1. Common statistical and machine learning methods

Principal component analysis (PCA) and linear discriminant analysis (LDA) are the most frequently employed multivariate data analysis techniques for fruit origin authentication (Appendix A). PCA is a common exploratory method mainly used as an initial step in pre-classification analysis to reduce data dimension, visualize sample data, initially assess the similarities between categories, and discover hidden patterns in data (Brombin *et al.* 2022). LDA is a classification method that aims to maximize the ratio of between-class variance to within-class variance, thereby obtaining a linear combination of variables (Tang *et al.* 2011). In addition, hierarchical cluster analysis (HCA), partial least squares discriminant analysis (PLS-DA) and its orthogonal variant (OPLS-DA) have also been used in fruit origin authentication as linear classifiers (Farag *et al.* 2022). The essence of HCA is to classify sample data, separate different data as far as possible, and gather similar data into a category (Cebi *et al.* 2017). The greatest advantage of PLS-DA is versatility, particularly when dealing with unanticipated scenarios in classical statistics, for example, when the number of variables greatly exceeds the sample size (Chen *et al.* 2020).

The most popular machine learning techniques used in fruit origin authentication include *k*-nearest neighbor (*k*-NN), support vector machines (SVM), artificial neural network (ANN), and random forests (RF). *k*-NN is a distance-based nonparametric classification method with simple, easy, and low-cost algorithms, which can well handle small data sets and multi-class problems (Maione and Barbosa 2019; Peng *et al.* 2019). By abstraction, simplification, and simulation, ANN may mimic the essential properties of human brains. It is renowned for its high capability of solving complex problems (e.g., image processing), as well as its simple design and easy implementation. The most often used ANN algorithm is back-propagation-artificial neural network (BP-ANN), which consists of input, hidden, and output layers. The performance of BP-ANN model is affected by many parameters, such as the number of hidden layer nodes, momentum factor, and initial weights (Shao *et al.* 2021). SVM achieves the classification of samples by mapping the data space into a high-dimensional space and finding the spatially optimal hyperplane that minimizes the error rate of the training set to maximum classification interval (Maione

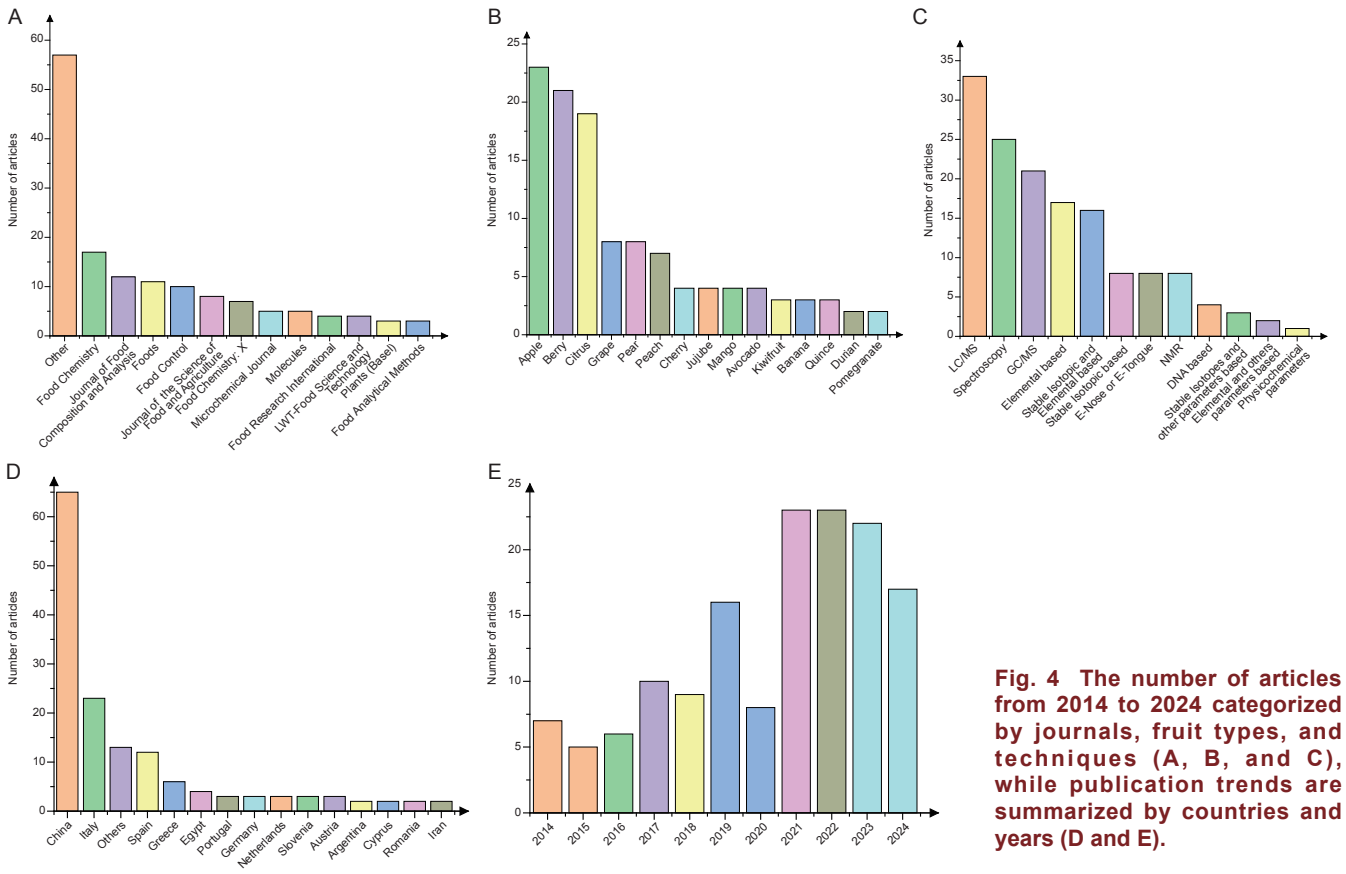


Fig. 4 The number of articles from 2014 to 2024 categorized by journals, fruit types, and techniques (A, B, and C), while publication trends are summarized by countries and years (D and E).

and Barbosa 2019; Xu *et al.* 2019). SVM can effectively prevent overfitting, which improves its ability to build models in training sets using many variables and relatively few experimental samples (Deng *et al.* 2020). RF is an ensemble model combining predictions based on regression trees and classification trees (De Santana *et al.* 2019). RF has advantages such as not requiring variables to follow a specific statistical distribution, a small number of training samples, low sensitivity to overfitting, and ability to prioritize attributes (Sun *et al.* 2018; Maione and Barbosa 2019). In addition, stepwise quadratic discriminant analysis (S-QDA), multilayer perceptron (MLP), multi-task residual fully convolutional network (MRes-FCN), extreme gradient boosting (XGBoost), C5.0, and convolutional neural network (CNN), are also used to identify the geographical origin of fruits (Maione and Barbosa 2019).

3.2. Mineral element fingerprint technology

Mineral elements are key elements involved in biological metabolism and also fundamental constituents of organisms (Zhang *et al.* 2019). Organisms must obtain mineral elements from the environment since they are unable to synthesize mineral elements themselves (Tyler and Olsson 2001). In general, mineral elements in orchards are derived from the atmosphere, water, soil and its parent material. Mineral elements can be intentionally added to fruits by atmospheric deposition, irrigation, fertilization, and pesticide application (Shuai *et al.* 2022a). Plants absorb mineral nutrients mainly through their roots from the soil to promote normal growth. Therefore, mineral elements in plants are related to soil characteristics. The elemental composition of agricultural products has the potential to be used in geographical origin authentication, as the elemental fingerprint is strongly influenced by mineral elements in the soil (Murphy *et al.* 2000).

Detection methods of mineral elements There are several methods for detecting mineral elements in fruits, and atomic absorption spectroscopy (AAS) is the most widely used approach, which includes graphite furnace-AAS (GF-AAS) and flame-AAS (F-AAS). Compared with F-AAS, GF-AAS requires a smaller sample volume and typically has a detection limit of 10^{-6}–10^{-5} mg kg^{-1}, making it suitable for both trace analysis and identification of viscous and solid specimens (Nie *et al.* 2024). In GF-AAS, samples undergo complete atomization inside the graphite furnace, improving atomization efficiency and detection sensitivity. Some other methods for specific elements can only detect one or two specific elements, such as hydride generation atomic fluorescence spectrometry (HG-AFS), fluorescence spectrophotometry (FS), dithizone colorimetry method (DCM), silver salt method (SSM), liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS), liquid chromatography-AFS (LC-AFS), and AAS (Nie *et al.* 2018). In practice, DCM and SSM are rarely used, and AFS is used for detecting volatile elements such as arsenic, mercury, and selenium (Nie *et al.* 2018).

Currently, ICP-MS is the most extensively utilized elemental analysis method that employs high-temperature plasma as an ion source (Nie *et al.* 2024). Characterization and quantification of elements are carried out based on element-specific mass (mass-to-charge ratio, m/z) and external standards, respectively (Zhang *et al.* 2018). ICP-MS has a larger measuring range, a higher measurement efficiency, and a lower detection limit than other instruments. As a highly potent analytical approach, ICP-MS allows quantitative determination of metal and nonmetal elements at low concentrations, generally at parts per billion (ppb) or parts per trillion (ppt) levels (Muñoz-Redondo *et al.* 2022). In addition, ICP-MS can detect both solid and liquid samples, which is superior to ICP-atomic emission spectroscopy (ICP-AES) (Muñoz-Redondo *et al.* 2022). The simultaneous multi-element determination of ICP-MS is superior to the unit-by-unit determination of AAS. Moreover, determination of the stable isotope ratio is a unique feature of ICP-MS, and ICP-MS coupled with LC can be used for speciation analysis of elements such as inorganic arsenic (Nie *et al.* 2018). Inorganic elements in fruits are often combined with organic matter to form compounds that are difficult to dissociate. In addition, organic matter often interferes with the determination of inorganic elements in the fruit. Therefore, in the determination of these inorganic elements, the organic matter must be first decomposed to release target components in forms suitable for measurement (Muñoz-Redondo *et al.* 2022). The most often used sample digestion methods for the determination of mineral elements in fruit by ICP-MS include dry digestion, wet digestion, microwave digestion, and pressure tank digestion (Nie *et al.* 2018). At present, microwave digestion is the most extensively used method for determining mineral elements in fruits (Zhang *et al.* 2018). In addition to dry digestion, blank tests are also required when performing sample digestion (Rechcigl and Payne 2008).

Use of mineral element fingerprinting in fruit geographical origin authentication Mineral elements are an important indicator for origin traceability of plant-derived agricultural products. From 2014 to 2024, there were 32 research articles related to mineral element-based geographical origin identification technology for fruits (Fig. 4-C). To date, mineral elements have been successfully applied to the identification of geographical origin of various fruits, such as apple (Zhang J Y *et al.* 2023), blueberry (Kuang *et al.* 2021), citrus (Hu *et al.* 2014; Hidalgo *et al.* 2023), jackfruit (Debbarma *et al.* 2021), lemon (Gaiad *et al.* 2016), pear (Coelho *et al.* 2019), strawberry (Strojnik *et al.* 2022), and winter jujube (Kong *et al.* 2024). Researchers from different countries have explored the use of ICP-MS to identify the geographical origin of local fruits with PDO status. Gaiad *et al.* (2016) used ICP-OES/ICP-MS to determine the main trace element composition in lemon samples from four producing areas in Corrientes Province, Argentina. Four models, including LDA, *k*-NN, PLS-DA, and SVM, showed good performance in identifying the geographical origin of lemon samples. Debbarma *et al.* (2021) applied ICP-MS to determine the 24 characteristic elements in both soil and jackfruit samples from different regions in India. These results showed that element analysis combined with PCA and LDA models can effectively distinguish the

geographical origin of jackfruit samples. Zhang *et al.* (2019) and Kuang *et al.* (2021) used ICP-AES/GF-AAS to determine elements in apple samples and blueberry samples from different origins in China, respectively. Based on 14 elements, the established LDA (prediction rate of 70.0%) and BP-ANN (prediction rate of 81.0%) models could effectively authenticate the geographical origin of apple samples (Zhang *et al.* 2019). Analysis of variance (ANOVA) and Duncan's multiple comparison test revealed significant differences in B, Ca, Cu, Fe, K, Mg, Mn, P, and Zn in blueberry samples from different regions, and the best multilayer perceptron neural network achieved a discriminant accuracy of 94.7% (Kuang *et al.* 2021). Mineral elements can also be used to identify the geographical origin of fruits from geographically small regions. For example, Hu *et al.* (2014) used ICP-MS to determine the mineral elements (Ce, Dy, Er, Eu, Gd, Ho, La, Lu, Nd, Pr, Sm, Tb, Tm, Y, and Yb) in navel oranges from different regions of Jiangxi Province in China. They suggested that the concentration and composition of mineral elements in navel orange peel could be used to identify the geographical origin of navel orange fruit from three major producing areas.

Factors affecting mineral element content in fruit The concentration of mineral elements may reflect the geographical origin of agricultural products (Tyler and Olsson 2001). Mineral elements originate from the natural diffusion of elements in rock, move through the soil, and finally reach the plant. Therefore, plant organs and tissues depend on the soil for growth. The efficiency of plant element uptake is an important factor affecting the content of mineral elements in plants (Muñoz-Redondo *et al.* 2022). This efficiency is largely dependent on the cation exchange capacity of the soil, soil pH, and soil fungi (Zhang *et al.* 2019). Therefore, soil directly affects the type and quantity of mineral elements in fruits. In addition, anthropogenic factors, such as soil management, fertilizer application (Nziguheba and Smolders 2008), and irrigation methods (Bryla *et al.* 2005) may also serve as key geographical parameters for the origin traceability of agricultural products.

In the study of the relationship between mineral elements in soil and jackfruit samples, Debbarma *et al.* (2021) revealed a clear correlation between mineral elements in the fruit and soil. Additionally, 24 mineral elements were identified as key variables to differentiate the geographical origin of jackfruit samples. Another study investigated the effects of three influencing factors (harvest time after maturity, storage time, and fertilizer application) on the mineral composition of kiwifruit samples (Santoni *et al.* 2016). The results showed that 23 mineral elements were significantly different under different harvest time, storage durations, and fertilizer application. For instance, the harvest period was positively correlated with the content of Ca, Mg, and P; the mineral element content and composition of the kiwifruit samples were significantly influenced by application of 125–250 kg ha^{-1} nitrogen and 200 kg ha^{-1} potassium in the orchard.

Other factors, including variety and harvest season, also affect the mineral element content in fruits. For example, Medveckienė *et al.* (2022) determined the mineral element content and composition in rosehip fruit flesh of different varieties and maturity stages. The results showed that maturity stage and variety have important effects on the mineral elements. The contents of Al, Ca, Mg, Mn, and Ti reached their maximum at stage I, while the contents of B, Cr, Cu, Fe, K, and P peaked at stage IV. Furthermore, the *Rosa canina* variety showed a higher mineral element content than other varieties.

3.3. Stable isotope fingerprint technology

Isotopes include stable and radioactive isotopes. Stable isotopes are naturally existing elements and are non-radioactive, containing one or two extra neutrons but having the same chemical properties as the element. Isotopic fractionation is the basic principle underlying the traceability of stable isotopes. Natural fractionation (changes in the ratio of "heavy" and "light" isotopes of an element) occurs in stable isotopes through a series of cycles, such as crystallization and melting, diffusion and transfer, evaporation and condensation, and absorption and desorption. Stable isotopic composition is expressed as delta notation (δ) and can be calculated with the following equation: $^{ij}\delta E$ (‰)=($^{ij}R_{sample}$–$^{ij}R_{reference}$)/$^{ij}R_{reference}$×1,000, where i and j are the heavier and lighter mass numbers of element E, and R_{sample} and $R_{reference}$ are the heavy-to-light ratios of element E in the sample and reference material, respectively. The isotopic delta value, expressed in ‰, reflects the ratio of heavy-to-light isotopes in a sample compared to a reference material (Li *et al.* 2022).

Detection methods of stable isotope ratio Elemental analyzer-isotope ratio mass spectrometry (EA-IRMS) is generally used to measure the stable isotopic ratio in samples. EA is an automated specimen preparation device that transforms specimens into simple gases that can be analyzed by IRMS (Shuai *et al.* 2023a). Compound specific stable isotope analysis (CSIA) is a relatively new method for determining the geographical origin of food, which was proposed in 1990 by Hayes *et al.* (1990). Compared with bulk stable isotope analysis, CSIA has higher sensitivity and accuracy in geographical origin authentication, tracing, and certification of food (Liu *et al.* 2023). The most widely used methods for CSIA are liquid chromatography combined with isotope ratio mass spectrometry (LC-IRMS) and gas chromatography-combustion furnace-isotope ratio mass spectrometry (GC-C-IRMS) (Elsner and Imfeld 2016). The coupling of GC or LC with IRMS allows precise measurement of stable isotopes within organic compounds (Elsner and Imfeld 2016). CSIA requires the preoperative extraction, purification, and concentration of all samples. In addition, the analytical conditions (such as extraction time) may also affect the detection of different compounds. Based on the location, agricultural activity, and natural biological processes in ecosystems, molecular-level ratios of different components can provide specific details regarding the chemical composition of food. Therefore, separation of components is an essential process to ensure the accuracy of analysis (Liu *et al.* 2023).

Use of stable isotope fingerprinting for geographical origin authentication of fruits In origin tracing of fruits, the common stable isotopic indices include C ($^{13}C/^{12}C$), O

($^{18}O/^{16}O$), H ($^{2}H/^{1}H$), N ($^{15}N/^{14}N$), S ($^{34}S/^{32}S$), and Sr ($^{87}Sr/^{86}Sr$) (Mahne *et al.* 2018; Shuai *et al.* 2023a). In the past few years, EA-IRMS has been used to determine the authenticity and geographical origin of various fruits, such as apple (Brombin *et al.* 2022), banana (Wang *et al.* 2020), grape (Mei *et al.* 2024), peach (Yang *et al.* 2022), strawberry (Strojnik *et al.* 2022), and sweet cherry (Wang *et al.* 2024). The differences in stable isotope values in several studies were compared to determine whether the stable isotope values differ among different origins. The δ^{2}H values measured in apples from different regions of Italy by Aguzzoni *et al.* (2021) and Mimmo *et al.* (2015) ranged from –66.7‰ to –55.5‰ and –26.07‰ to –24.96‰, respectively. The δ^{2}H values measured by Anh *et al.* (2022) of apples from the USA ranged from –111.57‰ to –84.60‰, while those of apples from Australia ranged from –27.22‰ to –25.81‰. Another study on apple found the δ^{2}H values of –100.1‰ in the United States, –45.0‰ in New Zealand, and –50.2‰ in China (Anh *et al.* 2023). The δ^{2}H value varies considerably between different countries (Anh *et al.* 2023). For δ^{18}O, according to the measurement from Anh *et al.* (2022), the δ^{18}O values of apple ranged from –5.24‰±0.74‰ to –0.74‰±1.62‰ in Australia, and ranged from –12.85‰±3.61‰ to –7.23‰±1.54‰ in the USA. According to the measurement from Anh *et al.* (2023), the δ^{18}O of apples was –10.5‰ in the USA, –8.8‰ in New Zealand, and –1.0‰ in China. In general, δ^{18}O and δ^{2}H values in different countries are of great importance for the identification of apple growing areas (Anh *et al.* 2022; Anh *et al.* 2023). Stable isotope values are effective in identifying the origin of fruits. However, a single stable isotope value often shows no significant difference across regions. Therefore, current studies have combined two or more stable isotopes to authenticate the geographical origin of fruits. For example, Kornexl *et al.* (1996) studied the stable isotopes of orange samples from America, Israel, Australia, Argentina, Spain, and Brazil. They found that the samples from different countries could not be distinguished by using δ^{13}C or δ^{15}N values alone, but could be discriminated by combining δ^{13}C with δ^{15}N values. Suzuki *et al.* (2012) successfully used δ^{13}C and δ^{18}O values to effectively distinguish apple samples from China and Japan, as well as samples from different origins in Japan.

Amino acids, disaccharides, fatty acids, monosaccharides, organic acids, and volatile substances are the main targets of CSIA (Wu H *et al.* 2022). At present, CSIA has been adopted in the geographical origin authentication of fruits. For instance, Meng *et al.* (2019) revealed that HS-SPME-GC-IRMS is efficient in determining the geographical origin of wolfberries. The accuracy of LDA models based on the δ^{13}C value of volatile compounds (limonene, tetramethylpyrazine, safranal, geraniol, and *β*-ionone) could reach 89.16%, 87.77%, and 85.87% for three origins of wolfberry samples, respectively. CSIA has also been adopted in other applications, such as identifying the adulteration of fruit juice (Guyon *et al.* 2014), but it has been rarely used in the geographical identification of fruits. As techniques for tracing the geographical origin of agricultural products, isotopic techniques still have certain limitations. For instance, soils in different production areas may have similar chemical compositions. Isotopic fractionation may also occur during fruit growth or processing, leading to the loss of isotopic information during analysis (Gamboa-Delgado 2021). Therefore, it is necessary to integrate different technologies, as the use of only isotopic techniques can hardly identify the geographical origin of fruits from similar regions. Over the past few years, several studies have demonstrated that stable isotope ratio combined with multi-element analysis may be a powerful tool to trace the geographical origin of fruits (García-Ruiz *et al.* 2007; Li A *et al.* 2021; Liu X H *et al.* 2021; Muñoz-Redondo *et al.* 2022). To further improve the accuracy of fruit origin authentication, some researchers have established a "stable isotope-element" fingerprint, which combines stable isotope analysis with mineral element analysis.

A "stable isotope-element" fingerprint could be used to identify the origin of fruits from various countries. Muñoz-Redondo *et al.* (2021) found that δ^{15}N, δ^{18}O, δ^{34}S, As, Ba, Cd, Cs, Pb, and Se, could serve as significant variables to classify mango samples from Brazil, Côte d'Ivoire, Equatorial Guinea, Mexico, Peru, Senegal, and Spain. Furthermore, the combination of stable isotopes and elements could achieve more precise results than the use of stable isotope or element alone. Muñoz-Redondo *et al.* (2022) also found that δ^{2}H, δ^{13}C, δ^{15}N, δ^{18}O, and 46 elements could be used as key variables to classify avocado samples from Brazil, Chile, Colombia, Kenya, Mexico, Peru, South Africa, and Spain (Muñoz-Redondo *et al.* 2022). Moreover, the PLS-DA model makes it possible to distinguish Spanish avocados from non-Spanish avocados with an accuracy of 98%.

A "stable isotope-element" fingerprint could also be used to identify the origin of fruit from various areas or regions in the same country. Liu X H *et al.* (2021) determined stable isotopes (δ^{13}C and δ^{18}O) and 15 mineral elements in juice of apple samples from different counties in China. The PCA, PLS-DA, and LDA models for regional classification of samples showed satisfactory performance (Liu X H *et al.* 2021). δ^{13}C, δ^{18}O, B, Cr, and Sr, are important indices for the traceability of the geographical origin of apple samples. The values of δ^{13}C, δ^{18}O, B, Sr, and Cr were –26.20‰ to –24.62‰, 3.92‰ to 9.00‰, 5.12 to 14.36 μg L^{-1}, 0.013 to 0.199 μg L^{-1}, and 0.59 to 2.54 μg L^{-1}, respectively. Bin *et al.* (2022) found that a single stable isotope cannot completely classify all muskmelon samples from different regions in Xinjiang Uygur Autonomous Region, China. The classification accuracy of the LDA model based on stable isotopes and multi-element data for the geographical origins of muskmelon samples in Xinjiang could reach 100% in Altay, 91.9% in Hami, and 79.1% in other regions. Li A *et al.* (2021) found that PCA could distinguish peach samples from coastal and inland regions in China, which can be mainly attributed to δ^{2}H and δ^{18}O. In addition, S-LDA and OPLS-DA based on three stable isotopic ratios (δ^{13}C, δ^{2}H, and δ^{18}O) and 18 elements were used to clearly distinguish 150 peach samples from six different regions. Among these indicators, δ^{2}H, δ^{13}C, δ^{18}O, Na, Cd, V, Al, Be, K, Co, Cu, and Ag serve as the main indicators. Some researchers attempted to combine stable isotopes, mineral elements, and secondary metabolites to further improve the accuracy of geographical origin authentication of fruits. For example, Bat *et al.* (2012)

combined these three kinds of indicators to identify the origin of apple samples. The combination of $\delta^{18}O$, $\delta^{2}H$, Rb, and S (sulfur) could effectively distinguish the origin of apple samples from different regions (Alpine, Dinaric, Pannonian, and Mediterranean) in Slovenia.

Factors influencing stable isotopes in fruit Regional environment, climate, terrain, metabolic types, and other factors can lead to isotopic fractionation, resulting in different stable isotopic ratios in organisms from different regions (Fig. 3). Therefore, as the natural "markers", stable isotopes are suitable for the geographical origin authentication of fruits (Muñoz-Redondo *et al.* 2022). The carbon isotope of $^{13}C/^{12}C$ mainly depends on the photosynthetic pathway of the plant (C_3, C_4, and CAM), carbon fixation pathways during photosynthesis, environmental factors (temperature, precipitation, light, atmospheric CO_2, and atmospheric pressure), and physiological parameters (leaf stomatal conductance of CO_2 and intercellular carbon dioxide concentration) (Farquhar 1989). The environment and climate vary with regions, time period, and spatial scale, which often have interactive effects on soil respiration and finally affect $\delta^{13}C$ values in plants (Rapisarda *et al.* 2010). Therefore, the $\delta^{13}C$ values in plants can reflect the difference in ecological environment between different regions (Shuai *et al.* 2022a). In addition, changes in soil respiration and moisture pattern caused by global warming and greenhouse gases also have important impacts on $\delta^{13}C$ (Jonasson *et al.* 1997). With global warming, the depth of permafrost decreases in high-latitude and cold regions, which indirectly changes the depth of groundwater and soil water distribution. This will ultimately affect soil respiration, thereby affecting the $\delta^{13}C$ value in plants (Billings 1987).

The $\delta^{15}N$ signature in soils and plants is affected by geographical and climate factors, such as mean annual precipitation and temperature, sunshine, distance to the coast, chemical fertilizers, and soil conditions (Cecchetti *et al.* 2020). All these factors modulate the nitrogen atmospheric deposition in soils, nitrogen fixation by plants, nitrogen processing by microbes, and nitrogen loss to the environment (Shuai *et al.* 2023b). In addition, the $\delta^{15}N$ value in plants is mainly influenced by soil properties and agricultural practices, and is highly dependent on available nitrogen sources and anthropogenic factors (Cecchetti *et al.* 2020; Martínez-Gaitán *et al.* 2020). Previous studies have shown that the $\delta^{15}N$ value of organisms is generally within the range of 6.4‰–11.2‰, since soils are usually more enriched in $\delta^{15}N$ than atmospheric N_2. The use of organic or synthetic fertilizers can alter the $\delta^{15}N$ value of organic nitrogen in soils. The $\delta^{15}N$ value of synthetic chemical fertilizers is relatively low, which generally ranges from –3‰ to 3‰ (Li A *et al.* 2021). Low $\delta^{15}N$ values in plants might be a result of agricultural intensification, which limits the potential geographical discrimination of $\delta^{15}N$. However, it can provide useful information on the overall agricultural practices in different countries (Rapisarda *et al.* 2010).

During the growth of fruit trees, isotope fractionation generally does not occur during the transport of water from the roots to the above-ground part. However, a series of biochemical reactions occurring during transpiration and photosynthesis on the leaf surface can lead to isotopic fractionation (Shuai *et al.* 2023b). The fractionation of $^{2}H/^{1}H$ and $^{18}O/^{16}O$ is mainly attributed to evaporation, precipitation, and transpiration by plants. Latitude, altitude, and longitude have significant impacts on the geographical distribution of hydrogen and oxygen isotopes in global water. The $^{2}H/^{1}H$ and $^{18}O/^{16}O$ ratios in plants generally vary with distance from the ocean and altitude. The $\delta^{2}H$ and $\delta^{18}O$ in groundwater gradually decrease from areas near the sea to inland areas. After seawater goes through the meteorological cycle (such as evaporation, condensation, rainfall, and precipitation), the oxygen isotope in groundwater will undergo some changes with certain regional variations (Rapisarda *et al.* 2010). With the evaporation of water from the ocean near the equator to areas of higher latitudes and elevations, the cooler temperature will lead to a gradual depletion of heavy isotopes in rainfall (Gat *et al.* 2003). In general, the water stable isotope ratio is mainly affected by temperature at high latitudes, by both temperature and precipitation at middle latitudes, and by precipitation at low latitudes. Notably, soil respiration, water distribution pattern, regional climate, and monsoon type are important factors affecting $\delta^{2}H$ and $\delta^{18}O$ fractionation in plants (Billings 1987).

Strontium has four stable isotopes, including ^{84}Sr, ^{86}Sr, ^{87}Sr and ^{88}Sr. $^{87}Sr/^{86}Sr$ (denoted by $\delta^{87}Sr$) is commonly used to indicate the strontium isotopic composition. Strontium isotopes naturally exist in calcium-containing minerals, and strontium in soil is primarily derived from the weathering of bedrock. Therefore, with negligible influence of external factors such as climate and season, the strontium composition in plants is determined by the ecological environment, rock age, and soil composition (Rummel *et al.* 2010). The $\delta^{18}O$ and $\delta^{2}H$ values of plants vary little among regions with small climatic differences, and therefore the use of strontium stable isotopes is an effective strategy to trace the origin of plant products (García-Ruiz *et al.* 2007). The stable isotopes of sulfur are ^{32}S, ^{33}S, ^{34}S, and ^{36}S. The composition of sulfur isotopes in soils is not only related to bacterial action, but also to the geological environment, rainfall, and distance from the ocean. The $\delta^{34}S$ in the soil is affected by the geological characteristics, proximity to the coast, human activities, climate, deposition of sea spray (aerosolized seawater containing sulfates), and farm practices, which in turn affect the $\delta^{34}S$ value in tissues of plants (Pianezze *et al.* 2020). It is also affected by local agricultural production conditions such as fertilization (Trust and Fry 1992). Therefore, the isotopic composition of organic sulfur in organisms can provide useful information for geographical origin authentication.

3.4. Fingerprint technology of metabolites

Biological metabolites are broadly categorized into primary and secondary metabolites. The former include essential pathways for the survival of all organisms, such as those producing carbohydrates, amino acids, typical fatty acids, and nucleic acids. The latter comprises metabolites such as aroma substances, flavonoids, phenolic substances, and phytosterols (Shuai *et al.* 2022a). Variations in environmental conditions (such as soil type, soil features, temperature,

precipitation patterns, and sunlight) across different geographical regions can lead to distinct alterations in plant metabolic pathways, thereby generating unique metabolic profiles (Lester 2006). While the genetic background of plants establishes their fundamental metabolic pathways, environmental factors play a crucial role in modulating the expression and activity of these pathways (Tohge and Fernie 2009; Saito and Matsuda 2010). As a result, the same plant variety cultivated in different geographical locations may produce varying metabolite profiles, and these differences can serve as a distinctive "fingerprint" for identifying geographical origin (Goff and Klee 2006).

Detection methods of metabolites (1) Aroma volatiles. Aroma volatiles are characterized by complex composition, low content, high volatility, and great diversity. Hence, they are easy to be transformed into new substances during heat treatment in extraction, or to decrease or even disappear (Sarry and Gunata 2004). Therefore, it is necessary to develop appropriate extraction methods to completely extract volatiles for studying the fruit aroma itself. Common methods for the extraction of volatile substances include headspace (HS), static headspace (S-HS), solid-phase microextraction (SPME), solvent assisted flavor evaporation (SAFE), liquid-liquid extraction-solid-phase extraction (LLE-SPE), simultaneous distillation-extraction (SDE), steam distillation (SD), supercritical fluid extraction (SFE), and stir bar sorptive extraction (SBSE) (Zhai *et al.* 2022). Different methods have their own advantages and disadvantages in practical application, and should be chosen according to the specific purposes. After extraction, the methods used to identify the specific volatile components include gas chromatography-mass spectrometry (GC-MS), gas chromatography-ion mobility mass spectrometry (GC-IMS), comprehensive two-dimensional gas chromatography coupled to mass spectrometer (GC×GC-MS), quadrupole time-of-flight mass spectrometer (Q-TOF-MS), gas chromatography-mass spectrometry olfactometry (GC-MS-O), and gas chromatography-flame ionization-olfactometry (GC-FI-O) (Różańska *et al.* 2022). Besides qualitative and quantitative analysis of volatile substances, it is important to determine the contribution of each volatile substance to the aroma profile. GC-O can separate a single component from a multi-component volatile substance and perform olfactory analysis. The odor activity value (OAV), relative odor activity value (R-OAV), and GC-O can be used to screen the key odor-active compounds (Różańska *et al.* 2022).

(2) Non-volatile compounds. Solvent extraction (SE), ultrasonic extraction (UE), microwave extraction (ME), enzyme extraction (EE), and supercritical fluid extraction (SFE) are common methods for extracting non-volatile compounds. The methods for identifying the extracted non-volatile compounds include high performance liquid chromatography (HPLC) and ultra-performance liquid chromatography coupled with quadrupole-time-of-flight-mass spectrometry (UPLC-Q-TOF-MS). HPLC is widely used to separate, quantify, and analyze non-volatile compounds dissolved in sample mixtures due to its high sensitivity, robustness, efficiency, and repeatability. The most often used detectors for HPLC are MS, ultraviolet-visible (UV-VIS), diode array detector (DAD), fluorescence, and electrochemical detection (ED) (Zhu *et al.* 2020). When combined with MS, HPLC can identify and quantify several compounds in a single measurement. UPLC-Q-TOF-MS can provide greater resolution, higher sensitivity, and better peak repeatability compared with other metabolomics systems, allowing quick and simultaneous relative quantification or qualification of numerous chemicals (Zhang *et al.* 2020). In addition to chromatographic methods, spectral fingerprinting techniques, such as Fourier transform-infrared spectroscopy (FT-IR), near-infrared spectroscopy (NIR), nuclear magnetic resonance (NMR) spectroscopy, and hyperspectral imaging (HSI), are also used to qualitatively trace metabolites for geographical origin authentication of fruits (Longobardi *et al.* 2013).

Application of metabolite fingerprinting to geographical origin authentication of fruits (1) Odor profile analysis techniques. Aroma volatiles in fresh fruits encompass a diverse array of chemical compounds, including amino acids, carotenoids, esters, fatty acids, phenolics, alcohols, aldehydes, ketones, lactones, and terpenoids. Among these, terpenoids (particularly C_{10} monoterpenes and C_{15} sesquiterpenes) and fatty acid derivatives are the predominant aromatic volatile compounds that are synthesized and released by fruits, playing primary roles in conferring unique flavor characteristics to plants (Zabetakis and Holden 1997; Winterhalter and Rouseff 2002; Akakabe *et al.* 2014). Some volatiles in fruits have relatively high olfactory perception thresholds, making them difficult to detect by smell (Winterhalter and Rouseff 2002). The odor, overall flavor quality, and origin of fruits are largely determined by low-threshold compounds (Akakabe *et al.* 2014). Some volatiles may be shared by most fruits, while some volatiles are specific to one or a few fruits. However, only a few volatiles with defined quantity and odor threshold have been recognized to contribute to fruit flavor (Sarry and Gunata 2004).

HS-SPME-GC-MS integrates extraction, separation, quantification, and qualification to accurately analyze samples, and has been widely used to analyze flavor volatiles for identifying the origin of fruits in recent years. Giannetti *et al.* (2017) identified 118 volatiles in apple samples by HS-SPME-GC-MS. The established PLS-DA model could achieve an accuracy of 87% in differentiating apple samples from different regions. Ethyl acetate, isobutyl acetate, propyl propionate, Z-2-hexene-1-alcohol, acetate, ethanol, 1-hexyl alcohol, and d-limonene are significant indicators. Medina *et al.* (2019) and Guo *et al.* (2020) used HS-SPME-GC-MS to determine aromatic attributes of apple samples from different geographical origins. Medina *et al.* (2019) found that ethyl acetate, 2-methyl-1-propanol, ethyl caproate, and toluene could be used as distinguishing indicators. Guo *et al.* (2020) revealed that the S-LDA model based on aroma components and characteristics could achieve a prediction accuracy of 93.5% in differentiating the geographical origins of apple samples. Another study showed that the main contributors to the characteristic aroma of 'Fuji' apples are ethyl 2-methylbutanoate, 2-methylbutyl acetate, and hexyl acetate (Hadi *et al.* 2013). Some studies have demonstrated that esters are the most common volatiles released from apples (Bryla *et al.* 2002). Moreover, esters were also identified

as authenticity indicators to verify the geographical origin of apple samples. Farag *et al.* (2022) used SPME-GC-MS to study the aroma volatiles of 14 Egyptian mango samples from three different regions, and found that monoterpenes are the most abundant compounds in most mangoes. PCA model showed that Egyptian mangoes from all three sources were rich in terpenes, and contained abundant *β*-terpene and (Z)-geranylendone (Farag *et al.* 2022). Terpene hydrocarbons are the most significant category of chemicals discovered in mango, whose concentrations range from 16% to 90% (Hadi *et al.* 2013).

HS features simple operation, fast analysis speed, and low cost; more importantly, it does not require complex sample pretreatment. However, it can only capture a small amount of aroma volatiles in the sample, which limits its use in the analysis of trace volatiles. Therefore, researchers have also attempted to develop other methods to extract volatile compounds. In addition, among different fruit tissues, the epidermal tissues can produce more volatiles than the internal tissues. Metabolic activity or the abundance of fatty acid substrates largely determines the ability of the epidermis to produce flavor compounds (Bryla *et al.* 2002). Based on these theories, Figueira *et al.* (2020) applied needle trap microextraction (NTME)-GC-MS to study the volatile compounds from lemon peel samples. The HCA and OPLS-DA models based on volatile compounds could well distinguish different lemon peel samples. Volatile organic compounds, such as butene, *α*-pinene, *α*-thujene, and 1-butanol, could be used as geographical markers of lemons.

(2) Taste profile analysis techniques. The phytochemicals in fruits mainly include phenolic substances (such as flavonoids, anthocyanins, resveratrol, and soy isoflavones), carotenoids (such as carotene, lutein, and zeaxanthin), and plant sterols (such as sitosterol, stigmasterol, and daucosterol). Carotenes include *α*-carotene, *β*-carotene, and *γ*-carotene. The phytochemicals in fruits from different regions vary greatly and play a key role in shaping the quality of fruits. Phenols, flavonoids, and phytosterols are important secondary metabolites for determining the geographical origin of common fruits.

Non-volatile metabolite fingerprinting technology has been widely employed in the identification of the geographical origin of fruits from different countries. Bat *et al.* (2018) used primary and secondary metabolites to identify the origin of apple samples from five production regions of Slovenia (Alpine, Dinarik, Mediterranean, Pannonia, and Sub-Mediterranean). Some phenolic compounds, such as flavanols, flavonols, and antipolyglycoside were identified as markers for geographical origin authentication of apple samples. Cheng *et al.* (2022) determined the total phenolic, total flavonoid, and total anthocyanin contents of black wolfberry samples from four regions in China. Compared with total phenolics and flavonoids, total anthocyanins were found to be a reliable marker for identifying the geographical origin of black wolfberry samples. PCA based on HPLC data could explain more than 95% of the sample information, which could clearly distinguish the geographical origin of black wolfberry samples. Guo *et al.* (2017) conducted extensive research on the polyphenol content of kiwifruit samples from PDO regions in China. The S-LDA model demonstrated a prediction rate of 92.2% for the geographical origin of kiwifruit samples. The main phenolic compounds in kiwifruit samples are (−)-epicatechin, (+)-catechin, proanthocyanidins B_1, and caffeic acid derivatives, which could be used to achieve satisfactory identification of kiwifruit samples.

Many studies have indicated that phenolics could be used as chemical markers to distinguish the origins of most fruits, but a few studies have reached different conclusions (Guo *et al.* 2017). For example, Li Y H *et al.* (2021) identified the geographical regions of navel orange samples by using UPLC-Q-TOF/MS. PCA and OPLS-DA models based on seven metabolites could well differentiate the fruit of Gannan from that of other regions. Narirutin 4′-glucoside, sinapic acid, and farnesyl acetone are potential differential metabolites for the geographical origin authentication of navel orange (Li Y H *et al.* 2021). The application of secondary metabolites in geographical origin authentication of plant products is significantly limited by their dynamic nature and close association with plant development and metabolism. While these metabolite levels are intrinsically linked to plant growth, their continuous and unpredictable variations throughout development often lead to identical secondary metabolite profiles in the same plant species from different locations, complicating reliable geographical origin identification (Shuai *et al.* 2022a). Some researchers have attempted to combine metabolites with stable isotopes to identify the geographical origin of fruits. For example, Zhang *et al.* (2022) found that sorbitol, glucose, fructose, sucrose, δ^2H, and δ^{13}C could be combined to distinguish Fuji apple samples from the Bohai Bay, the Loess Plateau (LP), and the Northwest region in China.

(3) Odor/taste profile analysis techniques. Electronic nose (E-nose) and electronic tongue (E-tongue) are rapid detection techniques to study the volatile and non-volatile compounds in fruits, respectively. Some studies have used E-nose to distinguish the geographical origin of fruit samples. For instance, Ma *et al.* (2014) and Centonze *et al.* (2019) explored the feasibility and stability of E-nose to distinguish the geographical origin of kiwifruit and orange samples, respectively. Ma *et al.* (2014) found that S-LDA and S-IMCA could accurately identify kiwifruit samples from three Chinese PDO regions in Hunan, Shaanxi, and Sichuan provinces. Centonze *et al.* (2019) found that the SELECT/LDA model could provide the highest prediction ability in cross-validation and external validation with mean values of 97.8% and 95.7% for the volatile compounds in orange samples from three countries (Italy, South Africa, and Spain), respectively. Wu X Y *et al*. (2022) combined flash GC-E-nose with PLS-DA, S-LDA, and DT to recognize 34 apple samples from four geographical regions, and S-LDA achieved the best recognition rate of 88.2%.

Some studies also combined E-nose with E-tongue to identify the geographical origin of fruits. Wu *et al.* (2017) and Wang *et al.* (2019) combined E-nose and E-tongue to study the flavors of Chinese apple and wolfberry, respectively. Wu X Y *et al*. (2022) concluded that PCA, LDA, SVM, and PLS-DA models exhibited satisfactory identification performance for 126 apple samples from seven producing regions in

China. Wang *et al.* (2019) found that the LDA model could classify the wolfberry samples from different geographical origins with a total classification accuracy of 86.4%, 86.8% and 92.6%, respectively.

(4) Spectroscopic analysis techniques. Chromatographic techniques allow for the detection of the food composition and subsequent characterization, while spectroscopic techniques allow rapid differentiation of foods based on their composition. Spectroscopy combined with chemometrics has exhibited outstanding analytical performance for food authentication (Hong *et al.* 2019). The advantages of these techniques are low cost and little or no need for sample preparation prior to analysis.

IR is the most frequently used rapid detection technology for identifying fruit origins. Khanmohammadi *et al.* (2014) used NIR data in conjunction with a least squares SVM model to classify persimmon samples from seven regions in Spain, resulting in the prediction results of 50 samples from an independent dataset with an error rate of only two percentage points. Tian *et al.* (2020) used the variable iterative spatial contraction method and stepped regression (VISSA-SR) combined with vision-near-infrared hyperspectral imaging to identify the geographical origin of apples from different regions (Jingning, Luochuan, and Qixia). The SVM model results showed that the VISSA-SR with detrending spectral preprocessing has superior performance. The corresponding calibration and prediction accuracies were 100% and 97.14%, respectively, indicating its effectiveness in identifying the geographical origin of apples from different regions.

In addition to IR, some other spectroscopy techniques are also used in the origin identification of fruits, such as ^{1}H NMR, a spectroscopic method that determines the molecular structure by utilizing the NMR phenomenon (Pauli *et al.* 2012). In recent years, ^{1}H NMR has been successfully used to determine the geographical origin of fruits. For instance, Longobardi *et al.* (2013) used non-targeted ^{1}H NMR fingerprinting technology to differentiate sweet cherry samples from Romania and Italy (specifically Puglia). The PLS-DA model showed significant performance with a 94.6% prediction accuracy, while the LDA model had the best average recognition and cross-validation prediction ability (100.0%) for the geographical regions of sweet cherries. The metabolites with great impact on the classification performance of LDA model include malic acid, glucose, fructose, glutamine, and succinic acid. Lin *et al.* (2021) used NMR to distinguish the geographical origin of sweet orange samples from four main producing regions in China. They quantified 62 sugars, amino acids, organic acids, alcohols, ketones, and flavones in sweet orange samples using ^{1}H NMR. Their results showed that Hubei sweet oranges are rich in phenolic compounds, while Sichuan sweet oranges are rich in amino acids. In addition, the combination of ^{1}H NMR with PCA, PLS-DA, and OPLS-DA models successfully achieved classification of sweet orange from different geographical regions. ^{1}H NMR exhibits unique advantages in fruit identification, as it can eliminate the need for sample preparation and filtration, avoid the loss of trace elements, and provide rich information of the sample (Pauli *et al.* 2012). In general, ^{1}H NMR combined with multivariate statistical analysis can achieve higher accuracy and reliability of fruit geographical origin authentication, serving as an effective tool for determining the authenticity of fruit origin (Pauli *et al.* 2012).

Factors influencing secondary metabolites in fruits The production and accumulation of flavor compounds in fruits are affected by numerous factors, such as variety, storage, maturation stage, fertilization, environmental conditions during cultivation (sunlight, irrigation water sources, and fertilization), and post-harvest management (duration and storage temperature) (Lester 2006; Shimizu *et al.* 2021). The flavor of fruits changes continuously along with fruit growth (Hou *et al.* 2020). Therefore, the maturation of fruits is an important factor affecting their secondary metabolites. Li C B *et al.* (2021) revealed the formation of volatile organic compounds during the ripening of passion fruit. At the intermediate stage and ripening stage of passion fruit, there are significant increases in the accumulation of esters, alcohols, ketones, hydrocarbons, alkanes, and aldehydes, as well as in the expression levels of related genes (Li C B *et al.* 2021). Wang Y *et al.* (2022) identified 23 significant volatile compounds in *Docynia delavayi* fruit, including octanal, geranylacetone, and butyl acetate. Young *Docynia delavayi* fruit had fewer key volatile aromatic compounds than mature fruit. Hou *et al.* (2020) found that monoterpenoids and aldehydes increase while esters decrease in oranges with ripening; and sesquiterpenes showed a fluctuating pattern throughout the maturation process. Dou *et al.* (2020) found that 'BaXi' and 'GuangFen No. 1' bananas harvested in March contained abundant isobutylisobutyrate, isobutylbutyrate, and isobutylcaproate, whereas those harvested in September were rich in hexyl acetate, indicating that bananas harvested in March have more volatiles than those harvested in September.

Incompletely ripe fruits may exhibit higher storability and marketability, as well as lower wastage. However, they typically have weaker flavor due to the close relationship between ripeness and the synthesis of volatile compounds (Wang Y *et al.* 2022). Preferably, fruits are harvested at their optimum eating quality, which can maximize the volatiles for better aroma (Kader *et al.* 2003). As the fruit ripens, it releases different volatile compounds depending on the geographical conditions (Dixon and Hewett 2000). Therefore, when using aroma volatiles for geographical origin authentication of fruits, it is important to ensure consistent maturity of all fruit samples. Genotype and genetic composition are also important factors affecting fruit flavors (Liu W J *et al.* 2021; Mihaylova *et al.* 2022). Liu W J *et al.* (2022) found that the 'Hujinmilu' white peach variety contained high levels of (E)-2-nonene aldehyde, 1-butyl alcohol, and styrene, while the 'Jinyuan' yellow peach variety exhibited higher levels of (Z)-3-hexene acetate, caprylic aldehyde, nonyl aldehyde, and 3,5-octadiene-2-one, which contributed to stronger banana, citrus, and honey aromas. Mihaylova *et al.* (2022) revealed great differences in the relative quantities of 65 volatile compounds (aldehydes, esters, and fatty acids) in different varieties of peach samples.

4. Future and outlook

Instrumental analytical techniques combined with machine learning algorithms have demonstrated significant potential in tracing the geographical origin of fruits, as evidenced by the comprehensive discussion on recent research. However, several challenges persist in the identification and practical application of fruit traceability technologies, which need to be clearly highlighted to inform and guide future research efforts.

4.1. Different-scale regions

Accurate identification of agricultural product origins, including fruits, depends on the unique characteristics of their growing environments, such as climate, soil, and altitude. This uniqueness exhibits varying degrees of variability and consistency across different regions and scales. Large-scale regions, such as continents, countries, and provinces, which have greater variability in environmental conditions, tend to have higher accuracies in origin identification. For instance, single traceability techniques have been successfully applied to differentiate apples, sweet oranges, peanuts, salvia, and red seaweed from various provinces in China (Bat *et al.* 2018; Lin *et al.* 2021; Zhang J Y *et al.* 2023; Dai *et al.* 2024; Zhao *et al.* 2024; Gao *et al.* 2025).

However, traceability techniques based on large-scale regional analysis are limited to the identification of general geographical origins, and usually cannot pinpoint localized areas or specific production regions. For example, Zhang J Y *et al.* (2023) developed a model that could distinguish apple samples from the LP and non-LP regions of China, but could not differentiate between LP samples from Shaanxi, Shanxi, Gansu, and Henan. Similarly, Muñoz-Redondo *et al.* (2021) created a model for mango origin identification, which could only differentiate samples from Brazil, Côte d'Ivoire, Equatorial Guinea, Mexico, Peru, Senegal, and Spain, but could not discriminate samples from different cities within the same country. Geographically proximate small regions, such as cities, counties, and towns, often have similar climate, soil, and growing conditions, making it challenging to accurately categorize the sample origin (Peng *et al.* 2022). The slight differences in climate, soil, and water quality between small regions within the same province can lead to overlapping characteristics in instrumental analysis combined with machine learning for the traceability of crops. For instance, Jin *et al.* (2023) employed metabolite profiling combined with an SVM model to trace the geographical origin of Taiping Houkui tea within its core production regions, achieving a misclassification rate of 6.67% (Jin G *et al.* 2023). Similarly, Bin *et al.* (2022) employed elemental isotope techniques to identify melons from different regions of Xinjiang, achieving 100% correct classification for Altay but only 79.1% correct classification for other regions (Bin *et al.* 2022). In existing research, geographical origin identification has been mainly conducted at large regional scales. Future research should prioritize origin authentication of samples from key production areas and geographical indication-protected areas of fruit products.

4.2. Sample size

Sample size plays a pivotal role in determining the accuracy and reliability of geographical origin identification of crops. The representativeness of the data and the capacity to fully capture the origin characteristics are greatly influenced by the sample size. Larger sample sizes, which encompass the diversity of the origin, can significantly enhance the accuracy of the model. For example, Chen *et al.* (2022) developed a geographical origin authentication model for rice using data from different years and sample sizes (Chen *et al.* 2022). They found that the model based on a three-year sample size (n=75) could achieve an accuracy of 100.0%, which was 12.0% higher than that of the model based on a single-year sample size (n=32), demonstrating that larger and more comprehensive datasets can yield more representative and reliable identification results. In contrast, a study on the origin identification of Keemun black tea from core production areas collected only 16 and 20 samples from Dongzhi and Guichi, respectively (Ren *et al.* 2022). Such a limited sample size led to more random results by nature. Although 100% accuracy was achieved using an SVM model, the small sample size undermined the robustness and generalizability of the findings (Ren *et al.* 2022). This highlights the importance of adequate sample sizes to ensure the reliability and validity of origin identification studies.

Sample size is a factor positively correlated with the size of the production areas. When the production area is extensive, a larger sample size is required to adequately capture the geographical and environmental variations within the region. For instance, the LP encompasses a vast area, including parts of Gansu, Shaanxi, Shanxi, and the northwestern region of Henan Province. In their study, Zhang J Y *et al.* (2023) collected 522 apple samples to trace the origin of Fuji apples from LP and non-LP production areas. This sample size was significantly larger than that typically used for origin identification of smaller geographical regions (Giannetti *et al.* 2017; Medina *et al.* 2019; Brombin *et al.* 2022). By employing ICP-MS combined with machine learning, the origins of LP and non-LP Fuji apples were successfully identified. The optimized RF model achieved a training accuracy of 95.30% and a prediction accuracy of 97.29%. The inclusion of a broader range of geographical and environmental features improved the consistency between the training and test sets, enhancing the model's reliability (Zhang J Y *et al.* 2023).

Ensuring sample balance is a critical requirement for achieving robust and reliable results in origin identification of crops. It directly influences the performance of the model and the accuracy of identification outcomes. Taking American ginseng as an example, sample balance encompasses multiple dimensions, including quantity, soil type, storage conditions, cultivation methods, harvesting seasons, and varietal differences. A notable example is the study of Shuai *et al.* (2023b) where the number of American ginseng samples collected from the countries of origin (the United States and Canada; 25) was significantly smaller than that from the non-origin regions (China; 56). This imbalanced sample size introduced potential bias into the model, increasing the risk of misclassifying American ginseng samples into incorrect

origins (Shuai *et al.* 2023b). A well-balanced sample set, on the other hand, can better represent the true distribution of each region, enabling the model to more accurately capture and reflect regional differences. Future studies should prioritize rigorous collection of samples that account for multiple dimensions of diversity, representativeness, and balance to enhance the reliability and generalizability of origin identification models.

4.3. Integration of instrumental techniques

Different instrumental techniques can analyze the unique chemical fingerprints or physical characteristics of samples, offering diverse and highly accurate analytical approaches for origin identification. In origin identification studies, combining multiple instrumental techniques can yield complementary chemical information, resulting in a more comprehensive chemical fingerprint and thereby enhancing the accuracy of origin determination. For instance, MS provides insights into the molecular weight and structure of compounds, while NMR offers detailed information on molecular structure and functional groups. Jin *et al.* (2023) integrated ^{1}H NMR with GC-MS to determine the geographical origin of Taiping Houkui tea. They discovered that a single technique could only capture partial flavor information, whereas the highest classification accuracy (93.33%) was achieved through data fusion using an SVM model (Jin G *et al.* 2023). Similarly, Tomita *et al.* (2015) combined ^{2}D NMR with LC/MS to identify the geographical origin of apple samples. The presence of a compound is considered to be more credible when corresponding signals are detected in both MS and NMR. Compounds such as L-rhamnitol and sugar signals (including sucrose, glucose, fructose, aspartic acid, and 2-methyl malate) were identified using ^{2}D NMR and LC/MS. A PLS-DA model based on seven key substances demonstrated excellent discrimination performance between apple samples from Japan and New Zealand.

Usually, a single analytical technique cannot effectively discriminate chemically similar compounds or components in complex mixtures of crops. Hyphenated techniques, such as GC-MS or LC-MS, have enhanced detection sensitivity and improved ability to separate and identify individual components within complex samples (Guo *et al.* 2020; Farag *et al.* 2022; Lösel *et al.* 2024). For example, Lösel *et al.* (2024) employed high-resolution LC-MS to detect odd- and short-chain fatty acids, phospholipids, and other compounds in almonds (Lösel *et al.* 2024). An RF model based on these compounds achieved a discrimination accuracy of 88.8% in identifying the geographical origin of almonds from various countries, including Australia, Spain, Iran, Italy, Morocco, and the United States (Lösel *et al.* 2024). The use of coupled techniques also generates multi-dimensional data, which can reveal subtle differences between samples, thereby enhancing the discriminative power of the model. In another study, HPLC was coupled with GC-MS to distinguish PDO and non-PDO American ginseng (Shuai *et al.* 2022b). It was found that combining data from HPLC and GC-MS improved the accuracy of the RF model to 100%, achieving a 10.3 percentage point increase compared to that obtained using either technique alone (89.7% for both HPLC and GC-MS). In summary, the integration of multiple instrumental techniques provides more comprehensive, accurate, and reliable chemical information, significantly enhancing the effectiveness of origin identification. However, it is important to note that direct fusion of data from multiple sources can introduce a substantial amount of redundant information. Future research should focus on developing appropriate data processing methods tailored to specific data challenges to further improve the prediction accuracy and reliability.

4.4. Machine learning (ML)

In recent years, the application of ML to geographical origin traceability has been significantly advanced, providing robust technical support and driving substantial progress. ML techniques can extract intricate information about the characteristics of agricultural products that are closely associated with their origins. Through the optimization of traceability models, ML facilitates more precise identification and tracking of product origins, thereby enhancing the accuracy and reliability of geographical origin determination.

ML has demonstrated remarkable capabilities of differentiating agricultural products from various regions by leveraging single mineral elements, metabolites, and stable isotope techniques (Chung *et al.* 2022; Peng *et al.* 2023; Li Y T *et al.* 2024). For instance, Peng *et al.* (2023) employed GC-MS to analyze the volatile components of Wuyi Rock tea. By developing an MLP model based on 176 volatile features, they achieved a high accuracy of 92.7% in distinguishing Wuyi Rock tea from core and non-core production areas. Similarly, Chung *et al.* (2022) utilized IRMS combined with ML to determine the geographical origin of *Agaricus bisporus*. An SVM model achieved a perfect accuracy (100%) in identifying the regional origins of *Agaricus bisporus*. These studies highlight the effectiveness of ML in enhancing the precision and reliability of geographical origin traceability for agricultural products.

Spectral data-based conventional models demonstrate slightly lower sensitivity compared to chromatography and mass spectrometry techniques. However, ML enhances their performance, rendering them particularly suitable for the geographical origin traceability of agricultural products. For example, Silva *et al.* 2015) employed NIR combined with ML to identify the geographical origin of rice (Silva *et al.* 2015). By utilizing a *k*-NN model based on selected infrared wavelength data, they achieved an impressive accuracy of 93%–100% in distinguishing rice from northern and northeastern Thailand. Similarly, Schütz *et al.* (2022) applied FT-NIR integrated with an SVM model to classify grain maize samples from Ukraine, Slovakia, Spain, Peru, and the United States. Their results demonstrated that the SVM model could accurately classify grain maize from different countries with an accuracy of 95%. These studies highlight how the integration of spectral techniques with ML effectively mitigates the limitations of spectroscopy, such as lower sensitivity, while achieving high accuracy in determining the geographical origin of agricultural products (Han *et al.* 2024; Li Y C *et al.* 2024; Sun *et al.* 2024; Ding *et al.* 2025).

ML excels at learning and extracting meaningful patterns from large and complex datasets, enabling accurate prediction of new and unseen data. However, each ML algorithm possesses unique strengths and limitations, and the selection of an appropriate model significantly influences the training outcomes and performance (Jeong *et al.* 2023). Complex ML algorithms often demand substantial computational resources and large datasets, and they may not always be the optimal choice. Instead, the selection of an ML algorithm should be tailored to the characteristics of the experimental data. For instance, when working with stable isotope and elemental data, which typically involve limited sample sizes, simpler algorithms such as SVM and RF are often preferred due to their efficiency and robustness. Future research should focus on identifying and applying the most suitable processing methods for specific data challenges, ensuring optimal model performance and practical applicability.

4.5. Agriculture practices

The main agricultural practices involved in fruit production include bagging *vs.* non-bagging, chemical fertilizers *vs.* organic fertilizers, and protected cultivation *vs.* open-field cultivation. These practices make origin traceability more difficult. Furthermore, they may partially 'blur' or 'mask' the geographical characteristics typically associated with the fruit origin (Sharma *et al.* 2014; Gruda *et al.* 2019; Iqbal *et al.* 2021).

Application of bagging results in a highly similar appearance of the same fruit variety in different regions, and reduces the unique characteristics derived from natural environmental factors such as sunshine and temperature. Therefore, the identification methods based on physical appearance have rather limited effectiveness (Sharma *et al.* 2014). The composition and concentration of mineral elements are considered to be reliable indicators of fruit origin, as they can directly reflect the elemental profile of the local soil. However, bagging protects the fruit from rainwater, soil splash, and dust, thus reducing its exposure to the geochemical features of the cultivation environment. Furthermore, there are significant differences in flavor metabolites between bagged and unbagged fruits. Due to reduced natural light exposure, fruits often exhibit lower sugar and organic acid contents, and a reduction of the flavor compounds such as aromatic volatiles and phenolic substances (Wang Y Y *et al.* 2022). In summary, bagging can weaken the influence of important growing conditions, making origin identification more difficult and uncertain.

The application of chemical and organic fertilizers can significantly alter the elemental and isotopic fingerprint of soils. Specifically, long-term excessive use of chemical fertilizers can lead to soil pH changes, physical compaction, and mineral imbalance, thereby shifting the soil environment away from its original natural balance (Iqbal *et al.* 2021). In contrast, application of organic fertilizers, especially commercial products, can introduce external elements into the local soil environment. These changes in soil characteristics are reflected in the composition of the fruit (Fan *et al.* 2023). The δ^{15}N value of fruit, for example, is a key indicator for identifying nitrogen sources. Chemically synthesized fertilizers usually exhibit δ^{15}N values near 0‰, whereas organic fertilizers tend to be enriched in δ^{15}N, with values often exceeding +5‰ (Shuai *et al.* 2022b). As a result, fruits grown with different fertilizer types exhibit different δ^{15}N values. When the same fertilizer from a single manufacturer is widely used across different regions, fruits from these areas may have similar elemental and isotopic characteristics. Conversely, using different fertilizers within the same region can result in different chemical fingerprints in fruits from the same origin (Rapisarda *et al.* 2010), which can affect the accuracy of models that use stable isotopes and mineral elements to trace the origin of fruits. In summary, the use of chemical and organic fertilizers can affect soil chemistry and introduce external elemental and isotopic information. This can cause incorrect classification of fruits from different origins as fruits from the same origin, or fruits from the same origin as fruits from different origins, thereby significantly affecting the accuracy of origin discrimination (Liu *et al.* 2020).

Protected cultivation (such as in greenhouses or tunnels) and open-field cultivation have a differential influence on the climate signals reflected in fruits. Under open-field conditions, characteristics such as the maturation period, sugar content, acid content, skin texture and metabolite composition of fruit can accurately reflect local climate conditions, including day-night temperature variation, rainfall and sunlight. In contrast, protected cultivation isolates crops from the natural climate by creating an artificial environment characterized by higher temperature, elevated humidity, and reduced exposure to wind and rain (Gruda *et al.* 2019). Although this isolation is beneficial for fruit growth, it limits the fruit's ability to accurately reflect the true climatic conditions of its origin. For instance, in protected environments with reduced light intensity, the accumulation of photosynthetic products is reduced, which affects the formation of certain colors and flavors (Bian *et al.* 2015). The absence of natural rainfall also reduces mineral deposition on the surface of the fruit, altering its chemical composition. Furthermore, irrigation often relies on groundwater, which is different from the water isotopes found in regional rainfall (Shuai *et al.* 2023a). This disruption to the fruit's water isotope composition makes it difficult to determine the fruit geographical origin. Thus, protected cultivation limits the acquisition of information about the local climate from the fruit, particularly isotopic information, thereby restricting the accuracy of traceability methods based on geographical origin. These changes not only mask the effects of natural climatic factors such as temperature and sunlight, but also influence the internal metabolic activities, making it a challenge to determine the fruit geographical origin using stable isotope techniques (Akimasa *et al.* 2018).

In summary, agricultural practices can introduce non-geographical variations, which often mask the original geographical characteristics of the product, thereby making origin traceability more difficult. Achieving more precise origin identification will require technical innovation to reduce the impact of agricultural practices. Enhancing the effectiveness of geographical identification techniques is essential for ensuring the reliability of traceability.

5. Conclusion

This review summarizes and discusses recent work applying multivariate data analysis techniques to identify the geographical origin of fruits from 2014 to 2024. For the geographical origin authentication of fruits, IRMS and ICP-MS can provide sensitive and reliable results. Spectroscopic methods also show great potential in determining the geographical origin of fruits. Furthermore, the data can be processed using supervised and unsupervised techniques. Notably, machine learning algorithms, such as ANN, *k*-NN, and SVM models, have exhibited great potential in fruit geographical origin authentication and may become more powerful tools in future research on fruit geographical origin discrimination.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

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