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Stable isotope and multi-element analysis coupled with DD-SIMCA for verification of Slovenian pork origin and Krškopolje pig authenticity

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A B S T R A C T

Pig farming in Slovenia has a long tradition and is known for producing high-quality pork; however, almost two-thirds is imported, raising concerns about mislabelling. In addition to modern Slovenian breeds (MB), the Krškopolje pig (KP), the only preserved autochthonous pig breed in Slovenia, is highly valued for its high-quality, marbled meat, which is sold at a premium price. This study presents the first Slovenian pork database on stable isotope (δ^2H, δ^{18}O, δ^{13}C, δ^{15}N, δ^{34}S) and elemental composition, comprising 148 samples: 107 authentic Slovenian pork samples (MB and KP), 22 foreign samples, and 19 samples labelled as Slovenian collected from supermarkets. We investigated the geographical origin of modern-breed pork and the breed authenticity of Krškopolje pig pork using DD-SIMCA class modelling. The established DD-SIMCA models showed high sensitivity (97 % and 96 %) and specificity (100 % and 89 %), respectively. When applied to commercial pork products labelled as Slovenian, 79 % of the samples exhibited isotopic and elemental profiles that differed from the authentic Slovenian reference class established in this study. These findings demonstrate the potential of combined isotope–elemental profiling coupled with DD-SIMCA modelling as a complementary tool for pork origin authentication and traceability verification. However, classification results indicate consistency with the reference class rather than legal origin status and should therefore be interpreted accordingly.

1. Introduction

Inconsistencies in meat quality and documented fraudulent practices within the red meat sector continue to raise consumer concerns regarding meat safety, authenticity, and origin. Consequently, robust and reliable labelling practices that clearly define the provenance and authenticity of meat products are essential (Ballin, 2010). Additionally, products from specific regions are often highly valued by consumers, which can lead to higher prices (Camin et al., 2007).

In Slovenia, livestock farming is the most widespread and important agricultural sector, dominated by cattle farming, followed by poultry, pig, sheep, horse production, as well as beekeeping (Government of the Republic of Slovenia, n.d.a). Swine farming in Slovenia has a long tradition, ensuring high-quality pork and pork-based products highly valued by consumers (Government of the Republic of Slovenia, n.d.a). Commercial pig breeds and hybrids commonly used in intensive pig production systems in Slovenia are hereafter referred to as modern breeds (MB), while the Krškopolje pig, the only preserved autochthonous breed, is of significant importance to breeders, consumers, and researchers. The breed is characterised by its black coat and a continuous white belt over the shoulders and forelegs, and is listed among endangered Slovenian farm animal breeds. It originates from the southeastern Dolenjska region, specifically the Krško–Brežiško field and the Gorjanci foothills. However, it is now widespread throughout Slovenia. It is valued for its high-quality, marbled meat (Tomažin, Batorek-Lukač, Škrlep, Prevolnik-Povše and Čandek-Potokar, 2019), and its ability to thrive under tough conditions (Papić, Šteferl, Plut, & Štukelj, 2025). Its meat is well-suited for roasting and traditional dried products such as dry-cured hams, salami, sausages, and pancetta. In 2017, the Association of breeders of the Krškopolje pig registered the

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trademark "Meat products from Krškopolje pig", available exclusively to members for products made entirely from the breed's meat (Tomažin et al., 2019).

Slovenia's pork self-sufficiency rate was only 37 % in 2023, resulting in substantial imports (Government of the Republic of Slovenia, n.d.a). This dependence on imported pork highlights the importance of reliable traceability and origin verification systems for products marketed as Slovenian. Robust authentication tools are essential for supporting accurate origin labelling, preventing food fraud, and maintaining consumer confidence.The purchase of locally produced meat supports environmental sustainability by reducing CO_2 emissions, strengthens regional economies, and contributes to agricultural self-sufficiency and the viability of small farms (Strojnik et al., 2022).

Verification of the geographical origin of meat is a growing concern, not only for consumers but also for government agencies overseeing meat quality (Nie et al., 2020). In the EU, food traceability is regulated by Regulation (EC) No 178/2002 (European Parliament & Council of the European Union, 2002), which defines traceability requirements across the food and feed chain. Specifically for pig meat, Regulation (EU) No 1337/2013 defines origin as meat derived from animals born, reared, and slaughtered in the same EU Member State or third country (European Commission, 2013). The interpretation of analytical authenticity assessments should therefore be considered in the context of current EU origin-labelling regulations. In Slovenia, additional national quality schemes are regulated by the Agricultural Act and apply exclusively at the national level (Government of the Republic of Slovenia, n.d.b). Although paper-based traceability and livestock tagging systems can, in most cases, verify the geographical origin of foods in the retail market, the food industry also needs methods to screen untargeted samples to confirm origin and prevent both intentional and accidental undeclared substitutions (Camin, Bontempo, Perini, & Piasentier, 2016; Zhao et al., 2023b).

Among the various analytical techniques available, stable isotope ratio analysis (SIRA) using isotope ratio mass spectrometry (IRMS) has emerged as an increasingly important tool for verifying the authenticity and traceability of foods of animal origin, supporting both the food industry and regulatory authorities (Camin et al., 2017; Zhao et al., 2023b). It is based on the transfer of isotopic signatures of light bioelements (δ^{2}H, δ^{13}C, δ^{15}N, δ^{18}O, δ^{34}S) from local feed and water into animal and plant tissues, creating an isotopic fingerprint linked to geographical origin and production practices. However, isotopic analysis alone is insufficient without a robust reference dataset comprising authentic samples that capture variability in geography, season, feeding regime, and production system (Kelly, Heaton, & Hoogewerff, 2005).

Stable isotope ratio and elemental analyses have been extensively applied to the authentication of animal-derived foods, including pork (Cristea, Voica, Feher, Puscas, & Magdas, 2022; Kim et al., 2017; Shin et al., 2018; Zhao et al., 2014, 2020), beef (Bontempo et al., 2023; Heaton, Kelly, Hoogewerff, & Woolfe, 2008), yak (Nie et al., 2020; Zong et al., 2024), lamb (Camin et al., 2007) and poultry. These studies have demonstrated that isotopic and elemental fingerprints, when coupled with multivariate statistical approaches, enable robust discrimination of the geographical origin of meat products (Vinci, Preti, Tieri, & Vieri, 2013).

Similarly, differences in feeding regimes and production systems across livestock species have been successfully characterised using stable isotope ratios and elemental composition, allowing discrimination among conventional, organic, and pasture-based systems in pork, beef, lamb, and poultry (Camin et al., 2007; Cristea et al., 2022; Park et al., 2018; Perini, Nfor, Camin, Pianezze, & Piasentier, 2021; Song et al., 2021; Vinci et al., 2013; Zhao et al., 2014, Zhao et al., 2020). In addition, isotopic and elemental profiling has been explored for breed discrimination in meat products, particularly in pork and beef (González-Martin, González-Pérez, Hernández Méndez, Marqués-Macias, & Sanz Poveda, 1999). For instance, the combined use of δ^{34}S and δ^{13}C isotopic signatures has been shown to discriminate both feeding regime and breed, enabling differentiation between Iberian and white pig breeds.

More recently, one-class classification approaches have been proposed as an alternative to traditional discriminant methods, which require predefined classes and force all samples into existing categories. Data-driven soft independent modelling of class analogy (DD-SIMCA) defines an acceptance region around a target class, allowing effective identification of authentic samples and rejection of non-conforming or unknown samples (De Andrade et al., 2024; Rodionova, Titova, & Pomerantsev, 2016; Zontov, Rodionova, Kucheryavskiy, & Pomerantsev, 2017). Such approaches are particularly suitable for food authenticity studies, where non-authentic samples are often poorly defined, highly variable, or insufficiently represented in the training dataset (De Angelis, Summo, Pasqualone, Faccia, & Squeo, 2024; De Oliveira Costa et al., 2024). In addition, DD-SIMCA reduces the risk of forced assignment of unknown samples to predefined classes and enables theoretical estimation of misclassification errors, which improves the reliability of authenticity assessment (Rodionova et al., 2016; Zontov et al., 2017). In the past five years, DD-SIMCA has been successfully applied to various foodstuffs, including fruits, vegetables, fish, coffee, tea, and sweeteners (Candeias et al., 2025; De Andrade et al., 2024; Fechner et al., 2024; Strojnik et al., 2022). Although comparison with other supervised classification methods could provide additional insight into model performance, the primary objective of this study was authenticity verification based on an authentic Slovenian reference class, for which DD-SIMCA represented a suitable and well-established approach in food authenticity research.

To date, no studies have used stable isotope or elemental analysis to authenticate Krškopolje pig meat. Existing research has instead focused on production system effects, including comparisons between organic and conventional rearing in terms of gene expression (Fazarinc et al., 2020; Tomažin, Batorek-Lukač, Škrlep, Prevolnik-Povše, & Čandek-Potokar, 2019), physicochemical and sensory properties of Krškopolje pig meat and fat, fermented products (Savić, Škrlep, Marušić Radovčić, Petričević, & Čandek-Potokar, 2025; Škrlep, Čandek-Potokar, Batorek-Lukač, Tomažin, & Flores, 2019), and gut microbiota composition (Papić et al., 2025).

Given the absence of authenticity studies for Krškopolje pig meat, the present study aimed to: (i) establish a reference database of stable isotope ratios of light elements and multi-elemental composition for Slovenian pork meat, including modern breeds (MB) and the autochthonous Krškopolje pig (KP); (ii) develop a DD-SIMCA one-class classification models to verify the geographical origin of pork from MB and breed authenticity of the Krškopolje pig (KP) meat; (iii) assess the declared Slovenian origin of pork meat available on the domestic market; and (iv) differentiate between organic and conventional feeding regimes of the Krškopolje pig (KP).

2. Materials and methods

2.1. Samples

One hundred and forty-eight pork samples (Fig. 1) collected between January and March in 2022, 2024, and 2025 were included in the study. All analysed samples consisted of *longissimus dorsi* muscle tissue obtained from slaughter pigs intended for meat production. Of these, 107 samples were obtained directly from farmers across four geographical regions of Slovenia to establish a representative database of authentic pork. This group comprised 35 samples from commercial pig breeds and hybrids representative of intensive Slovenian production systems (hereafter referred to as modern breeds; MB) and 72 samples from the Krškopolje pig (KP), including 35 conventionally and 37 organically reared animals originating from registered pedigree animals included in the national breeding programme. According to information provided by farmers, MB pigs were generally reared in intensive production systems with higher maize inclusion in feed formulations (approximately

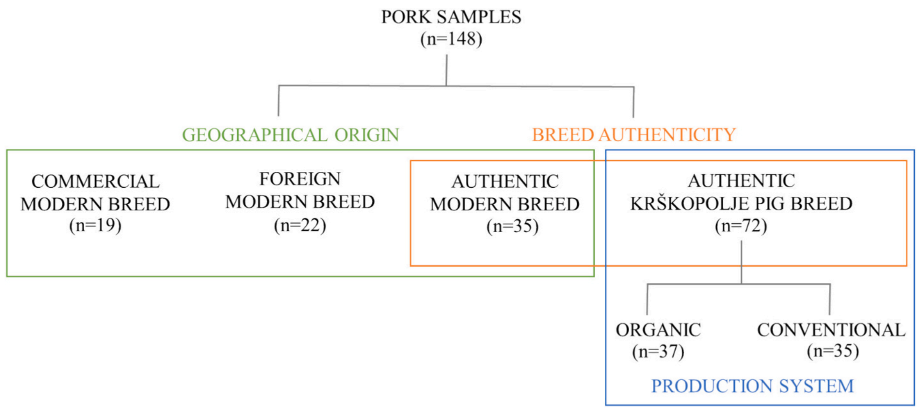

Fig. 1. Classification of pork samples (n = 148) according to geographical origin, breed and production system (conventional vs. organic).

40%), whereas KP pigs were more commonly raised in free-range or semi-intensive systems with lower maize supplementation (approximately 20%) and access to C_3 vegetation. Where available, additional metadata including slaughter weight, slaughter date, and production system were recorded. However, detailed zootechnical information such as age, sex, and complete production records was not consistently available for all samples. Additionally, the database included 22 non-Slovenian pork samples originating from several European countries, including Austria, Belgium, Croatia, Germany, Hungary, Italy and Spain. Due to the limited number of samples per country, these samples were treated collectively as a heterogeneous non-Slovenian reference group rather than as representative country-specific datasets. A further 19 commercially available samples labelled as Slovenian origin were also included. All samples were stored in plastic containers and kept at −20 °C before analysis.

2.2. Sample preparation

From each sample, a known weight (500 g) of meat was removed using a ceramic knife and homogenised in a blender fitted with a titanium blade. For water extraction, the obtained meat juice was filtered through GF/PVDF 45/25 filters (Macherey-Nagel, Chromofil, Germany) and stored at 4 °C until analysis. The homogenate portion intended for stable isotope and multi-element analysis was transferred to a plastic container and freeze-dried (Liof Gamma 1–16 LSC plus, Christ, Germany).

The removal of meat fat was performed according to the method described by (Potočnik et al., 2020), with some modifications. Lipids were removed using a solvent extraction procedure prior to isotopic analysis. Although residual lipid content was not quantified after extraction, all samples were treated using the same protocol and extraction conditions to ensure comparability among samples. Briefly, 4.5–5.0 g of lyophilised meat sample was defatted using 25 mL of a petroleum benzine: diethyl ether mixture (2:1, *v*/v). The mixture was homogenised using an Ultra-Turrax homogeniser for 3 min at 1200 rpm, followed by centrifugation (Type Centric 322 A, Tehtnica, Slovenia) for 5 min at 3200 rpm. This procedure was repeated three times. The supernatant was then removed, and the tube containing the pellet was placed in a water bath at 33 °C for 1 h to facilitate solvent evaporation. The pellet was then rinsed twice with Milli-Q water (Millipore, USA) and centrifuged at 3200 rpm. Finally, the defatted samples were freeze-dried and stored in plastic container.

2.3. Stable isotope and elemental analysis

2.3.1. Stable isotope analysis

Stable isotope ratios of light elements were determined using isotope ratio mass spectrometry (IRMS) and expressed in the δ-notation in ‰ according to eq. (1) (Brand et al., 2014):

$$\delta\left({}^{i/j}E\right) = \delta^{i/j}E = \frac{{}^{i/j}R_P - {}^{i/j}R_{\mathrm{Ref}}}{{}^{i/j}R_{\mathrm{Ref}}} x1000 \tag{1}$$

where superscripts i and j denote the higher and the lower atomic mass number of element E, R_p and R_{Ref} represent the ratios between the heavier and the lighter isotope ($^2H/^1H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{34}S/^{32}S$) in the sample (P) and in the reference material (*Ref*), respectively. δ^2H and $\delta^{18}O$ are reported relative to the V-SMOW (Vienna-Standard Mean Ocean Water) standard; $\delta^{13}C$ values are reported relative to the V-PDB (Vienna-Pee Dee Belemnite) standard; while $\delta^{15}N$ and $\delta^{34}S$ are reported relative to AIR, and the V-CDT (Vienna Cañon Diablo Troilite) standard, respectively.

The $\delta^{18}O$ values were determined both in lyophilised samples (bulk) and in extracted water, because these measurements reflect different isotopic sources. While $\delta^{18}O$ in solid tissue integrates oxygen derived from diet and metabolic processes, $\delta^{18}O$ of extracted water more directly reflects the isotopic signature of environmental and drinking water sources.

2.3.2. Determination of δ^2H and $\delta^{18}O$ values in solid samples

To analyse the lyophilised (defatted powder) samples, approximately 0.2 mg of material was weighed into silver cuspules for δ^2H_{solid} and $\delta^{18}O_{solid}$ determination. The $^2H/^1H$ and $^{18}O/^{16}O$ ratios were measured using the IRMS (Finnigan DELTA XP, Thermo Scientific) coupled to an elemental analyser (TC/EA; Finnigan DELTA TC/EA, Thermo Scientific). The international reference standards used were as follows: for $^2H/^1H$, keratins CBS (Caribou Hoof Standard; δ^2H = −157.0 ± 0.9 ‰) and KHS (Kudu Horn Standard; δ^2H = −35.3 ± 1.1 ‰), were used all obtained from the U.S. Geological Survey; for $^{18}O/^{16}O$, benzoic acid IAEA-601 ($\delta^{18}O$ = 23.14 ± 0.19 ‰) and benzoic acid IAEA-602 ($\delta^{18}O$ = 71.28 ± 0.36 ‰) both from the IAEA. Each sample was analysed in triplicate. The reproducibility was ±0.5 ‰ for $\delta^{18}O$ and ± 3 ‰ for δ^2H.

2.3.3. Determination of $\delta^{18}O_{wat}$ in extracted water samples

Briefly, 200 μL of the sample was purged with CO_2 at 40 °C for 3 h. Once equilibrated, the oxygen isotopic composition was measured using an IRMS (IsoPrime 100, IsoPrime, Cheadle, UK), connected to a Multi-Flow Bio preparation system (Multi-Flow; IsoPrime, Cheadle, UK). Data were normalised against USGS53 ($\delta^{18}O$ = 5.47 ± 0.03 ‰) and laboratory reference material seawater ($\delta^{18}O$ = 0.36 ± 0.04 ‰), and snow ($\delta^{18}O$ = −18.91 ± 0.03 ‰). A laboratory reference material (Milli-Q water: $\delta^{18}O$ = −9.12 ± 0.04 ‰) was used as quality control. The expanded uncertainty (k = 2) for $\delta^{18}O$ in water was ±0.5 ‰.

2.3.4. Determination of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values

A known weight (5 mg) of each lyophilised (defatted powder) sample and 5 mg of WO_3 were transferred to a tin capsule. Determination of stable isotope ratios of light elements, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{34}S/^{32}S$, was performed simultaneously for each sample using an IRMS equipped with a preparative module for solid samples (IsoPrime100 - Vario PYRO Cube EA/CNS Pyrolyser/Elemental Analyser). All measurements were normalised against the following reference materials USGS89 ($\delta^{13}C = -18.13 \pm 0.11$, $\delta^{15}N = 6.25 \pm 0.12$ ‰ and $\delta^{34}S = 3.86 \pm 0.56$ ‰); USGS88 ($\delta^{13}C = -16.06 \pm 0.07$ ‰, $\delta^{15}N = 14.96 \pm 0.14$ ‰ and $\delta^{34}S = 17.10 \pm 0.44$ ‰); USGS42 ($\delta^{13}C = -21.09 \pm 0.10$ ‰, $\delta^{15}N = 8.05 \pm 0.10$ ‰ and $\delta^{34}S = 7.84 \pm 0.25$ ‰). Other reference materials included CRP-CASEIN ($\delta^{13}C = -20.3 \pm 0.09$ ‰, $\delta^{15}N = 5.62 \pm 0.19$ ‰, $\delta^{34}S = 4.18 \pm 0.74$ ‰); and IAEA-600 caffeine ($\delta^{13}C = -27.77 \pm 0.04$ ‰, $\delta^{15}N = 1.02 \pm 0.05$ ‰). Each sample was analysed in triplicate, and the mean values were calculated. The reproducibility was ±0.2 ‰ for $\delta^{13}C$, and ± 0.3 ‰ for both $\delta^{15}N$ and $\delta^{34}S$.

2.3.5. *Elemental composition*

Approximately 0.15 g of the lyophilised sample was weighed into a precleaned Teflon tube to which 1 mL of nitric acid (HNO_3 65% Suprapur® for trace analysis, Supelco®) was added. Samples were subjected to microwave digestion using a Single Reaction Chamber Microwave Digestion System (ULTRAWAVE, MILESTONE, Italy). The program was as follows: heating to 240 °C in 20 min, then held for 15 min. The digested solution was quantitatively transferred into a polyethene graduated tube and diluted to 10 mL using Milli-Q water (Merck, Millipore, Watertown, MA, USA). Before analysis, samples were additionally diluted with 5% HNO_3 in a ratio 1:5 and 1:10. Determination of selected elements (Na, Mg, Al, P, S, K, Ca, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Rb, Sr, Mo, Ag, Cd, Sn, Sb, Cs, Ba, Hg, Tl, Pb) were performed on an inductively coupled plasma triple quadrupole mass spectrometer (ICP-QQQ, Agilent 8800, Agilent Technologies, Tokyo, Japan). Concentrations of the selected elements were expressed on a freeze-dried (lyophilised) weight basis.

Quantification was achieved using external calibration. Blanks and selected reference materials (Mussel tissue NIST 2976 and ERM-CE278k, Typical diet NIST 1548a and 1548b, Bovine Muscle BOVM-1) were also prepared in the same way as the samples. Every tenth sample was prepared in duplicate. Elemental limits of detection (LoD) were calculated as three times the standard deviation of the blank. Method accuracy was evaluated using certified reference materials. Limits of detection (LoDs) ranged from 0.1 ng/g for Cd, Cs, Co and Tl to 23 μg/g for Ca (Table 1). The mean recovery across all measured elements was 97.6 ± 5.9%, demonstrating good agreement between measured and certified values. Element-specific recoveries ranged from 87.9 ± 12.5% (Ag) to 109.3 ± 3.5% (Se), confirming satisfactory analytical performance.

2.4. *Statistical evaluation*

Data visualisation was conducted using box plots and dual-isotope scatter plots generated in Python (Version 3.13.1) and the Matplotlib library. Box plots were used to visualise and compare the distributions of isotopes and elements across different countries, breeds, and rearing systems. Dual-isotope scatter plots (δ^2H vs. $\delta^{18}O$; $\delta^{13}C$ vs. $\delta^{15}N$) were used to investigate geographical trends in isotopic composition and to compare the isotopic signatures of the analysed pork samples with reference values reported in the literature for other countries.

The Kruskal-Wallis test, a non-parametric method, was used to identify statistically significant differences ($P < 0.05$) among pork samples using the XLSTAT software package (Add-insoft, New York, USA). In addition, a non-parametric Mann–Whitney test was applied for pairwise comparisons between KP and MB groups, with significance set at $P < 0.05$. For multivariate statistical analysis, only elements consistently detected above the LoD in more than 30% of the total samples were used, meaning Ni and Ag were removed from the dataset (See Supplementaary Material 2; Table S1). Each value was divided by the LoD, and the natural logarithm of the result was taken (Mahynski, Strojnik, Shen, & Ogrinc, 2025). This procedure was applied to normalise the data and facilitate outlier detection.

Table 1
Limits of detection (LoD) and recoveries obtained for the determination of elemental concentrations in certified reference materials analysed by ICP-MS.

Element	LoD (μg/g or ng/g)	Recovery (%)	SD (%)
Na	8.4 mg/g	92.7	8.8
Mg	0.5 mg/g	92.2	5.4
Al	0.14 mg/g	103	4.6
P	1 mg/g	91.7	6.3
S	6 mg/g	93.6	8.7
K	9.6 mg/g	94.4	5.9
Ca	23 mg/g	91.9	4.2
Cr	4 ng/g	93.0	6.0
Mn	7 ng/g	99.2	1.6
Fe	0.33 mg/g	95.0	2.2
Co	0.10 ng/g	93.7	7.4
Ni	50 ng/g	96.9	6.0
Cu	20 ng/g	96.9	6.0
Zn	0.5 mg/g	100	3.7
As	0.6 ng/g	104	2.9
Se	2 ng/g	109	3.5
Rb	6 ng/g	103	2.5
Sr	4 ng/g	102	4.5
Mo	1 ng/g	97.6	2.2
Ag	0.2 ng/g	87.9	12.5
Cd	0.1 ng/g	104	4.7
Sn	0.7 ng/g	109	3.2
Sb	0.2 ng/g	96.3	6.7
Cs	0.1 ng/g	88.8	4.0
Ba	7 ng/g	101	4.0
Hg	0.3 ng/g	98.3	1.6
Tl	0.1 ng/g	106	8.8
Pb	0.4 ng/g	92.7	6.9

Orthogonal partial least squares discriminant analysis (OPLS-DA) was performed on the stable isotopes and multiple elemental data using SIMCA-P (version 17.0.2, Umetrics, Malmö, Sweden) software package, as described in (Strojnik et al., 2022) using default internal 7-fold cross-validation (1/7 CV). Model performance was evaluated using the explained variance (R^2Y) and predictive ability (Q^2), which were used to assess the quality of the model. To assess model robustness permutation testing ($N = 200$) and CV-ANOVA were used. Variables contributing most to class separation were identified using Variable Importance in Projection (VIP) scores, with VIP > 1 considered indicative of significant discriminant variables.

DD-SIMCA analyses were conducted using the modified DD-SIMCA MATLAB GUI tool (Zontov et al., 2017) alongside the mdatools package in R version 4.0.3 (Kucheryavskiy, 2020) as described by (Strojnik et al., 2022) and (Mahynski et al., 2025). The modelling approach is based on principal component analysis (PCA), where an acceptance region for the target class is defined using score distance (SD) and orthogonal distance (OD). The number of principal components was selected based on minimisation of classification error and model stability. For model validation, we used a k x m nested cross-validation strategy to repeatedly split the data in different ways, accounting for uncertainty arising from sampling error associated with small training sets (Mahynski et al., 2025). In each iteration, samples from the target set were randomly divided into training and test sets to assess model validity (sensitivity). This repeated hold-out approach balances sufficient data for model construction with reliable independent testing. Information from the nontarget samples was used only to evaluate the model's performance in terms of specificity (Strojnik et al., 2022). Those samples were not used during model building; therefore, the external validation was fully independent. Sensitivity and specificity were used as figures of merit. Sensitivity denotes the proportion of correctly identified samples of the target class. At the same time, specificity is the proportion of objects of the alternative class correctly identified as members of that class. The sensitivity can be defined as 100 (1– α) % and the specificity as 100 (1– β) % (Fidelis et al., 2017; Rodionova et al., 2016). In addition to sensitivity and specificity, the DD-SIMCA models were evaluated using total efficiency (*TEFF*), defined as *TEFF* = (Sens + Spec)/2.

3. Results and discussion

All the data presented in the paper and supplementary material 2 (Table S1) are part of the IsoFoodTrack database developed by Jožef Stefan Institute (JSI; (Terro et al., 2025). Measurements of $\delta^{18}O_{solid}$ and $\delta^{2}H_{solid}$ were not performed for all samples.

3.1. Regional discrimination of Slovenian pork meat

3.1.1. Stable isotope data

Although Slovenia is one of the smaller European countries, it is characterised by pronounced environmental heterogeneity resulting from the interaction of Alpine, Dinaric, Pannonian, and sub-Mediterranean climatic and geological regions (Hamzić Gregorčič et al., 2020). These regions differ substantially in altitude, temperature, precipitation patterns, hydrology, soil properties, vegetation, and agricultural practices, all of which may influence isotopic signatures in animal tissues. In particular, δ^{2}H and δ^{18}O values primarily reflect the isotopic composition of local meteoric water and are affected by altitude-related depletion effects, regional climatic conditions, and water sources (Hobson & Koehler, 2015). The measured δ^{2}H values of Slovenian pork ranged from −121.9 ‰ to −101.2 ‰ (mean − 109.8 ‰), while δ^{18}O values ranged from 8.7 ‰ to 11.6 ‰ (mean 10.4 ‰), and $\delta^{18}O_{wat}$ values of extracted tissue water ranged from −6.8 ‰ to −4.3 ‰ (mean − 5.8 ‰) (Table S2). These values differ from values reported for regions of China and Tibet (Fig. 2A; Zhao et al., 2023a), likely reflecting differences in climate, hydrology, and water-use patterns. Regarding Europe, the $\delta^{18}O_{wat}$ values of Slovenian pork align closely with those reported for Germany (−7.1 ‰) (Thiem, Lüpke, & Seifert, 2005), Romania (−6.5 ‰), the Czech Republic (−5.7 ‰), and Spain (−5.2 ‰), and are lower than values typical of the Netherlands (−2.1 ‰) (Cristea et al., 2022). These observations support the use of hydrogen and oxygen isotopes as tracers of environmental background and geographical origin.

In contrast, δ^{13}C values primarily reflect feeding practices rather than environmental conditions (Bahar et al., 2008; Monahan, Schmidt, & Moloney, 2018; Nie et al., 2020). Slovenian pork exhibited δ^{13}C values ranging from −23.0 ‰ to −17.5 ‰ (mean − 20.0 ‰), consistent with mixed C_3/C_4 feeding regimes and varying levels of maize supplementation. Compared with literature data, Slovenian values fall within the range commonly reported for European production systems, where C_3-based feeds predominate, while pork originating from countries with higher maize inclusion rates, such as the USA, Mexico, Chile, and parts of China, generally exhibits less negative δ^{13}C values (Supplementary Material 2, Table S2; Fig. 2B). As these data originate from independent studies employing different sampling strategies, sample sizes, production systems, the figure should be interpreted as a qualitative comparison rather than a direct statistical evaluation among countries. Nevertheless, it provides useful context for positioning Slovenian pork within the broader isotopic landscape reported worldwide. Thus, δ^{13}C provides valuable information on feeding practices and may contribute to geographical differentiation when regional feeding systems differ substantially.

The interpretation of δ^{15}N values is more complex because nitrogen isotopic composition is influenced by multiple interacting factors, including fertilisation practices, manure application, protein sources in feed, and production systems (Nie et al., 2020; Piasentier, Valusso, Camin, & Versini, 2003). Slovenian pork displayed δ^{15}N values ranging from 2.6 ‰ to 7.4 ‰ (mean 4.6 ‰), representing a broader range than that reported for several European countries (Supplementary Material 2, Table S2; Fig. 2B). This variability likely reflects differences in farm management and feed sourcing rather than geographical location alone. While climatic conditions may also influence δ^{15}N values, management-related factors generally exert a stronger effect. The substantial overlap of δ^{15}N values reported across European countries indicates that nitrogen isotopes have limited discriminatory power as standalone indicators of geographical origin at the continental scale. However, distinct δ^{15}N signatures have been reported in specific environmental and production contexts, such as Canada (Park et al., 2018), suggesting that δ^{15}N can provide complementary information when integrated with other isotopic and elemental markers within a multivariate authentication framework.

Among the isotopic parameters investigated, δ^{34}S provides one of the most direct links to regional environmental characteristics because sulphur isotope composition is influenced by local geology, soil properties, groundwater, atmospheric deposition, fertilisation practices, and

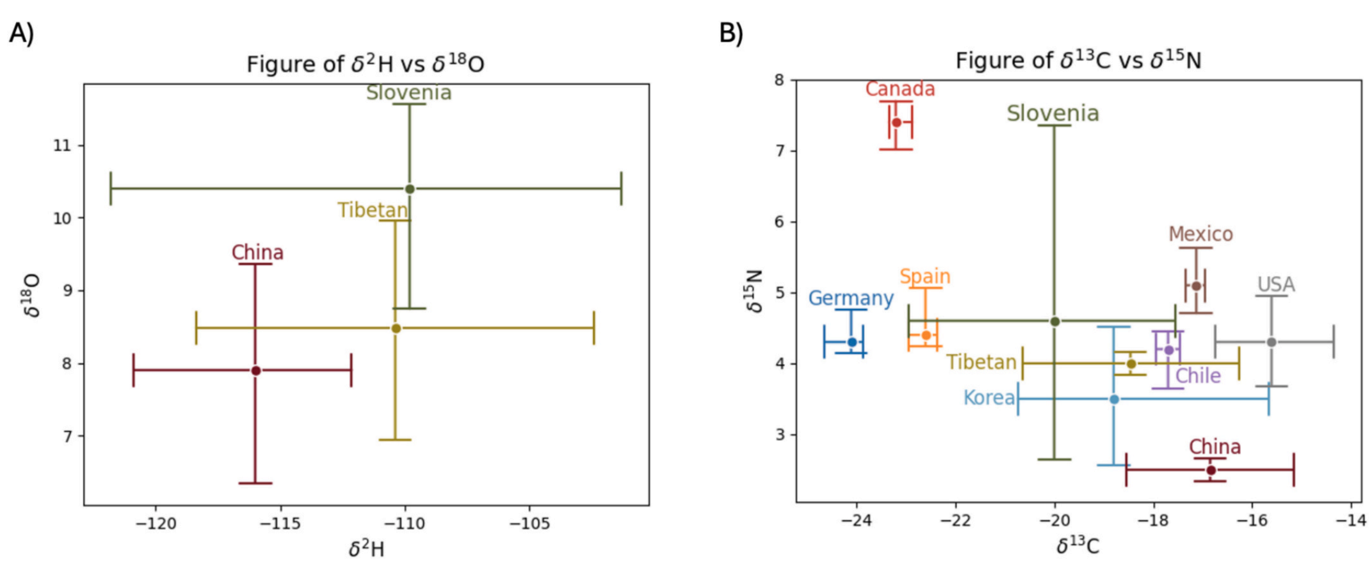

Fig. 2. (A) Representation of average, minimum and maximum values of δ^{2}H (x-axis) and δ^{18}O (y-axis) for authentic Slovenian pork samples, compared with published data for Tibetan pork and Chinese pork; (B) Representation of average, minimum and maximum values of δ^{13}C (x-axis) and δ^{15}N (y-axis) for authentic Slovenian pork samples and foreign samples. Position of the line represents the average and the range represents minimum and maximum values for various European and non-European countries (Park et al., 2018; Zhao et al., 2023a; Zhao et al., 2020).

proximity to marine environments (Sun, Kobayashi, Strosnider, & Wu, 2017). Slovenian $\delta^{34}S$ values ranged from 2.0 ‰ to 6.5 ‰ (mean 4.8 ‰), which is comparable to values reported for Spain, Belgium, and other terrestrial European production systems (Supplementary Material 2, Table S2). The observed sulphur isotope signatures indicate a predominantly terrestrial production environment with minimal marine influence. Similarly, yak meat from four Chinese regions exhibited a relatively narrow $\delta^{34}S$ range (2.7–6.3 ‰) (Nie et al., 2020), comparable to that observed in Slovenian pork. This similarity suggests that, despite their geographical separation, both datasets reflect predominantly terrestrial production systems and relatively homogeneous sulphur sources. In contrast, the substantial regional variation in $\delta^{34}S$ values reported for European lamb meat (1.6–12.8 ‰) by Camin et al. (2007) highlights the potential of sulphur isotopes as indicators of geographical origin. As $\delta^{34}S$ integrates information from environmental sources that are generally less affected by short-term management practices than other isotopic markers, it represents a particularly promising tracer for geographical origin authentication when used in combination with hydrogen, oxygen, carbon, nitrogen, and elemental composition data.

3.1.2. Multi-elemental composition

The elemental composition of meat is influenced by dietary factors, which in turn reflect the mineral characteristics of soil and drinking water, thereby linking elemental profiles to geographical origin (Ballin, 2010; Katerinopoulou, Kontogeorgos, Salmas, Patakas, & Ladavos, 2020). In Slovenian samples, mean concentrations of macro-elements followed: K > P > Na > Mg > Ca corresponding to 9.41–17.8 mg/g (K), 4.90–9.27 mg/g (P), 0.88–1.70 mg/g (Na), 0.54–1.07 mg/g (Mg), and 0.08–0.16 mg/g (Ca). This pattern is consistent with reports from South Korea (Song et al., 2021), Romania (Cristea et al., 2022), and Tibetan pork (Zhao et al., 2023a), reflecting the conserved physiological regulation of essential mineral elements in skeletal muscle. Unlike stable isotope ratios, which can respond directly to differences in feed composition and environmental conditions, the concentrations of major mineral elements are maintained within relatively narrow ranges through physiological homeostasis. Consequently, macro-element distributions showed substantial overlap among countries. Slovenian samples displayed somewhat higher median concentrations of K and Mg, whereas P, Na, and Ca values were largely comparable to those reported elsewhere.

The relatively elevated Mg concentrations observed in Slovenian pork may reflect a combination of environmental and nutritional factors. Slovenia is characterised by diverse geological formations, including carbonate-rich bedrock and mineral-rich soils, which can influence Mg concentrations in forage crops and locally produced feed ingredients (Hamzić Gregorčič et al., 2020; Potočnik et al., 2020). In addition, differences in feed formulation, including the use of locally sourced cereals, forage components, and mineral supplements, may contribute to the observed variation.

In contrast to macro-elements, trace elements are generally more sensitive to regional environmental conditions and therefore may provide stronger discriminatory information regarding geographical origin. Among these, Se, Rb, and Cs have been identified as particularly informative markers (Zhao et al., 2023a). Selenium concentrations in animal tissues are influenced by the natural Se content of soils, which varies considerably among geographical regions, as well as by feeding practices and dietary supplementation (Lee et al., 2006; Park et al., 2018; Zhao et al., 2020). Slovenian pork samples exhibited relatively low median Se concentrations with noticeable variability, whereas substantially higher concentrations were observed in Hungarian samples. Intermediate values were recorded for Italy, Spain, and the non-Slovenian reference group. These findings are consistent with previous studies demonstrating that both environmental and nutritional factors influence Se accumulation in animal tissues. Alkali elements, particularly Rb and Cs, are readily mobilised in soils and efficiently transferred through plants into animal feed and subsequently into animal tissues (Cristea et al., 2022; Kelly et al., 2005). Because Rb follows potassium during plant uptake and reflects the geochemical characteristics of soils and regional feed crops, it represents a particularly useful tracer of environmental background. Slovenian pork samples exhibited higher median Rb concentrations and greater variability than most other countries included in the study. Elevated Rb concentrations may reflect the geological diversity of Slovenia and the use of locally sourced feed ingredients originating from Rb-enriched agricultural soils. Previous Slovenian studies have similarly reported regional variability in elemental composition associated with geological and environmental differences (Hamzić Gregorčič et al., 2020; Potočnik et al., 2020). The importance of Rb as a geographical marker is further supported by Heaton et al. (2008), who identified Rb as one of the key variables for discriminating beef according to geographical origin. Although interpretation of country-specific differences should be made cautiously because of the limited number of samples available for several countries, the observed variability supports the role of Rb as a sensitive indicator of environmental and feed-related influences. Similar geographical differences have been reported for other trace elements, including Cu and Mn, which were found at elevated concentrations in pork from Vojvodina, together with higher Ca concentrations compared with pork from other regions (Tomović, Petrović, Tomović, Kevrešan, & Džinić, 2011). Overall, the results suggest that while macro-elements primarily reflect physiological regulation, selected trace elements retain stronger environmental signatures and therefore provide valuable complementary information for geographical origin authentication.

3.1.3. Chemometric modelling with the DD-SIMCA method

Isotopic and elemental data, including $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, and elemental concentrations of Na, Mg, Al, P, S, K, Ca, Cr, Mn, Fe, Co, Cu, Zn, As, Se, Rb, Sr, Mo, Cd, Sn, Sb, Cs, Ba, Hg, Tl, and Pb were used to build the model for Slovenian pork samples (Fig. 3a). Variables such as $\delta^{18}O$, $\delta^{2}H_{solid}$, Ag, and Ni were excluded from the final DD-SIMCA model either due to incomplete datasets or because they did not improve model discrimination performance during optimisation (Supplementary Material 2, Table S3). For the geographical origin model (Slovenian vs. non-Slovenian), two principal components were used with a significance level of $\alpha = 0.05$. The non-Slovenian samples were included as a heterogeneous external reference group intended to provide an initial evaluation of model robustness rather than a fully representative dataset of all possible foreign pork origins. The model achieved high classification performance, with a sensitivity of 97% in the training set and 100% specificity in the test set (Fig. 3b), resulting in a total efficiency (*TEFF*) of 98.6% (Supplementary Material 2, Table S3). Once validated, the DD-SIMCA model was applied to 19 supermarket samples labelled as Slovenian origin. The reported classification outcomes should be interpreted with caution due to the limited number of commercially available samples analysed in the present study. Each commercial sample was projected into the DD-SIMCA model space to assess its consistency with the Slovenian reference class. Fifteen samples were located outside the model acceptance region (green line, Fig. 3c), indicating isotopic and elemental profiles that differed from those characterising the authentic Slovenian reference dataset. Four samples fell within the acceptance region and were therefore considered consistent with the Slovenian reference class. Given the limited number of commercially available samples analysed in this study ($n = 19$), these results should be regarded as a proof-of-concept evaluation of the authentication framework rather than as a representative assessment of the Slovenian market.

Among the analysed commercial samples, six carried labels indicating that the animals were born, reared, and slaughtered in Slovenia. Four of these samples were classified within the Slovenian acceptance region, while two were located outside the acceptance region but close to the model boundary. The remaining thirteen samples were labelled only as being of Slovenian origin, without additional information regarding the place of birth, rearing, or slaughter. All of these samples were classified outside the Slovenian acceptance region.

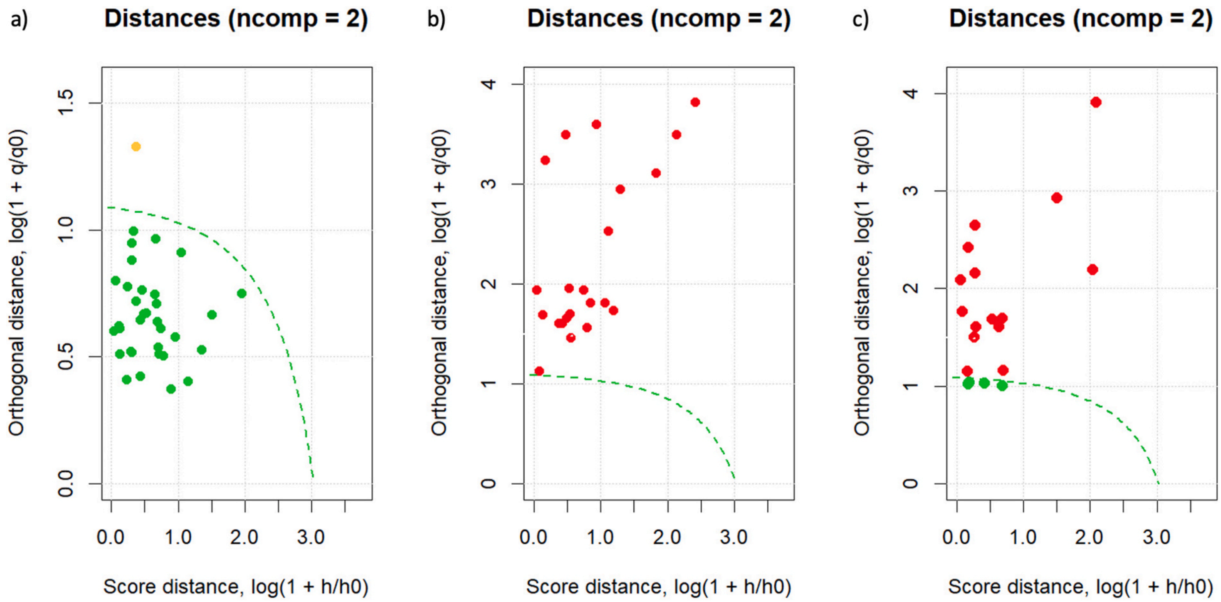

Fig. 3. DD-SIMCA classification model of (a) authentic modern breed Slovenian samples (green dots; $n = 35$), (b) non-Slovenian samples (red dots; $n = 22$), and (c) commercial samples from the market labelled as Slovenian ($n = 19$). The green dashed line represents the 100(1-α)% acceptance area, i.e., the decision boundary for whether the sample belongs to the target class based on α value of 0.05. Green points in a) indicate correctly identified authentic samples. The yellow point represents a misclassified authentic sample. Red points in b) denote correctly classified samples from abroad. In c), red points represent commercial samples that fell outside the model's acceptance region, indicating they are likely of non-Slovenian origin, and green points located within the acceptance region represent commercial samples consistent with Slovenian origin. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The classification results should not be interpreted as evidence of incorrect labelling or regulatory non-compliance. The reference model developed in this study represents pork originating from animals reared and processed entirely within Slovenia, whereas current EU legislation allows origin declarations under more complex supply-chain scenarios. Consequently, a classification outside the Slovenian acceptance region indicates that the isotopic and elemental profile differs from the established Slovenian reference class, but does not provide definitive evidence regarding legal origin status. Such differences may reflect alternative production histories, feed sourcing practices, or supply-chain configurations that are not represented in the current reference database.

Furthermore, the present model should be regarded as a proof of concept demonstrating the potential of isotopic and elemental profiling for pork authentication. Future work should focus on expanding the reference database across multiple production years, geographical regions, and production systems to further evaluate model robustness and support its long-term practical implementation. The independent non-Slovenian samples used in this study served primarily as an external validation set, while the commercial samples provided an initial assessment of the applicability of the approach under market conditions.

In recent years, the “Selected Quality” label has been introduced at the national level for various foods (e.g., dairy products, fruits, and vegetables) produced by certified producers, ensuring that the products are exclusively Slovenian. It now forms part of the national quality system, which enables consumers to recognise and select products produced in Slovenia, while also ensuring high standards (Government of the Republic of Slovenia, n.d.b). However, at the time of sampling, authentic Slovenian farms had either not yet been certified with the “Selected Quality” label or this information had not been recorded. In the future, this model could be valuable for verifying sample authenticity or for producers seeking certification.

3.2. Differentiation between Slovenian pig breeds

The aim of this part of the study was to evaluate whether stable isotope ratios and elemental composition allow discrimination between pure bread autochthonous Krškopolje pigs (KP), originating from registered pedigree animals included in the national breeding programme, and modern commercial pig breeds and their hybrids (MB) used in intensive production systems.

Statistically significant differences between KP and MB were observed for stable isotopes (δ^{13}C, δ^{15}N, and δ^{34}S), macro-elements (Na, K, P, Mg, S, and Ca), and several micro-elements (Fe, Ba, Cr, Cs, Mn, Rb, Se, Al, Sb, and Mo) (Supplementary Material 1, Fig. S1). These results indicate pronounced compositional differences between KP and MB samples, likely reflecting the combined influence of breed-related characteristics, feeding regimes and production environments.

In addition, an OPLS-DA model was applied to assess multivariate discrimination between the two breed groups. The OPLS-DA model (Fig. 4) demonstrated clear separation between KP and MB, with good explanatory and predictive performance, as indicated by $R^2Y = 0.674$ and $Q^2 = 0.611$, with a small difference between R^2Y and Q^2 suggesting low risk of overfitting. The OPLS-DA model was validated by permutation testing ($N = 200$) and CV-ANOVA. The permutation tests ($n = 200$) yielded R^2 intercept of 0.0965, and Q^2 intercept of −0.196. Both values are within accepted thresholds (R^2 intercept <0.3; Q^2 intercept <0), indicating that the models were not overfitted and that the observed discrimination is statistically significant. CV-ANOVA further confirmed the model's robustness, with highly significant P-values (< 6.3e-24) and large F-values (> 47). The prediction accuracy reached 92.8%. with class-specific accuracies of 95.8% for KP and 88.9% for MB. Variables with VIP > 1 identified Mg, K, P, S, Cs, δ^{15}N, Na, Se, and Rb as the most influential discriminators. These results suggest that the farming system has a stronger influence on the observed isotopic and elemental differences than the breed itself. This is supported by the variables identified as significant discriminators, which are consistent with a previous study by Zhao et al. (2020), where Ca, Mg, K, Na, Fe, and δ^{15}N were reported as key factors for differentiating pork samples obtained under conventional and organic feeding regimes. Indeed, the majority of MB samples (33 out of 35) originated from a conventional farming system, whereas KP samples came from both organic ($N = 37$) and conventional ($N = 35$) production systems. Therefore, the observed discrimination between KP and MB may partially reflect differences in production systems in

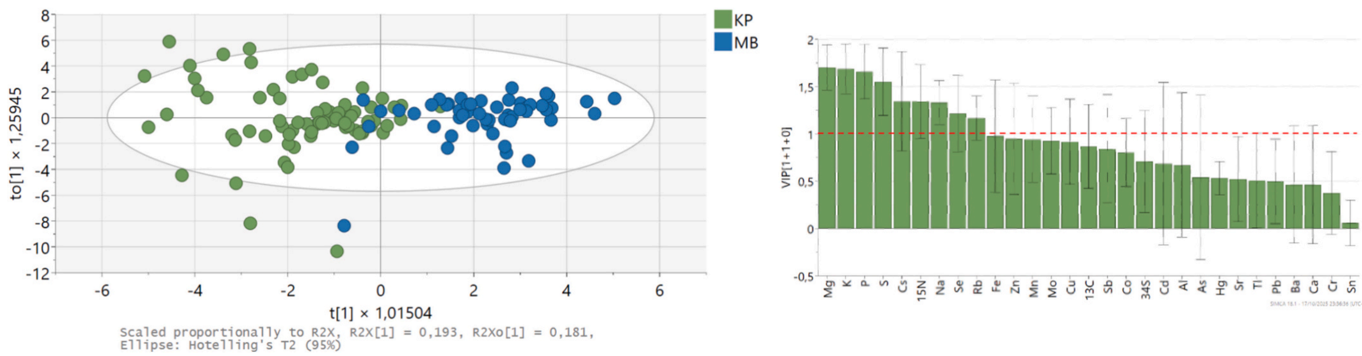

Fig. 4. OPLS-DA model differentiating between Krškopolje pig (KP) and modern (MB) breed based on isotopic ratios and elemental composition. Score plots (left) illustrate sample clustering by pig breed, with the 95% confidence interval ellipse. Variable Importance in Projection (VIP) plots (right) highlight the most influential variables (VIP > 1, red dashed line) for group discrimination. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

addition to breed-specific characteristics. This imbalance reflects the limited availability of organically reared modern pig breeds in Slovenia.

To eliminate the potential influence of the farming system on breed differentiation, the analyses were repeated using only samples from conventional production systems, including the Krškopolje pig (N = 35) and modern commercial breed samples ($N = 33$). Consequently, all results presented in Supplement Material 1, Fig. S1 refer exclusively to conventional production samples and do not include organically reared KP animals. Where available, slaughter weight data for conventional KP and MB animals indicated generally comparable slaughter ranges; however, age data and complete production records were not available for all samples. The Mann–Whitney test indicated that the same key variables remained highly significant ($P < 0.0001$), namely δ^{15}N, δ^{34}S, Mg, Al, P, S, K, Se, Rb, and Cs, together with additional variables showing significance at $P < 0.05$ ($\delta^{18}O_{wat}$, δ^{13}C, Na, Ca, Cr, Mn, Mo, Sb, and Hg). Compared with the full data, the new OPLS-DA model using only conventional production systems improved the separation between KP and MB, with good explanatory and predictive performance, as indicated by $R^2Y = 0.730$ and $Q^2 = 0.666$. The prediction accuracy reached 95.6% with class-specific accuracies of 94.3% for KP and 97.0% for MB. Variables with VIP > 1 identified Mg, K, Mg, P, S, Cs, δ^{15}N, Rb, δ^{34}S, and Na as the most influential discriminators.

The δ^{13}C values ranged from −23.0 ‰ to −17.5 ‰ (mean − 20.0 ‰) in conventional MB and from −25.9 ‰ to −15.7 ‰ (mean − 21.4 ‰) in conventional KP (Supplement Material 1, Fig. S1a). It can be observed that the δ^{13}C value of MB pork was generally higher than that of KP pork, likely reflecting differences in feeding composition between MB and KP production systems. Access to C_3 vegetation in KP production systems likely contributed to more negative δ^{13}C signatures (Bahar et al., 2008). Comparable observations were made by (Cristea et al., 2022), who reported δ^{13}C values ranging from −21.2 ‰ to −14.7 ‰ (mean − 18.0 ‰) in pigs reared in rural Romania, reflecting maize-rich (C_4) feeding regimes in the traditional household system.

The δ^{15}N values in conventional MB samples ranged from 2.6 ‰ to 7.4 ‰ (mean: 4.5 ‰), whereas KP samples ranged from 4.2 ‰ to 7.6 ‰ (mean: 6.0 ‰) (Supplement Material 1, Fig. S1a). KP samples generally exhibited slightly higher δ^{15}N values. Thus, the higher δ^{15}N values observed in KP pigs cannot be attributed to the inclusion of organically reared animals. Nitrogen isotopic composition in animal tissue is influenced by several factors, including feed composition, fertilisation practices, protein source, and production system characteristics (Monahan et al., 2018; Zhao et al., 2023b). Previous studies have shown that organically produced feed and manure-based fertilisation may contribute to elevated δ^{15}N values in animal products, although environmental and management-related factors may also influence isotopic variability (Erasmus, Muller, Butler, & Hoffman, 2018; Pianezze, Camin, Perini, Corazzin, & Piasentier, 2021). In the present study, conventional KP pigs were generally reared in more extensive production systems with lower maize supplementation and greater access to outdoor environments than MB pigs, which may have contributed to the observed isotopic differences. However, because detailed information on feed composition and fertilisation practices was not available for all farms, the relative contribution of these factors cannot be quantified. Therefore, the higher δ^{15}N values observed in KP samples most likely reflect a combination of feeding, management, and environmental influences rather than breed-specific effects alone.

The concentrations of macro-elements (Na, K, P, Mg, and Ca) were significantly higher in conventional MB samples than in conventional KP samples (Supplement Material 1, Fig. S1b). This difference likely reflects variations in feeding practices, as modern commercial pigs are typically fed highly formulated diets enriched in minerals, whereas Krškopolje pigs may receive more traditional or locally sourced feed. Similar trends have been reported in previous studies, where conventionally reared pigs exhibited higher concentrations of several macro-elements, including Na, K, Mg, and Ca, than animals raised under alternative production systems (Zhao et al., 2020). Sodium and potassium play essential physiological roles in maintaining osmotic balance, acid-base regulation, and nerve function, while calcium and magnesium are involved in skeletal development and numerous metabolic processes. Consequently, their concentrations in muscle tissue may be influenced by both dietary intake and physiological regulation (Zhao, Wang, & Yang, 2016). The observed differences observed between KP and MP samples likely reflect the combined effects of feed composition, mineral supplementation, production intensity and management practices. Although breed-related physiological differences cannot be excluded, the available data suggest that production system characteristics are likely the dominant drivers of the observed elemental variation.

3.2.1. Chemometric modelling with the DD-SIMCA method

A DD-SIMCA model was constructed following a similar approach as for geographical origin authentication. The model was built using samples of the Krškopolje pig (KP) as the reference class and externally validated with samples of the Slovenian modern breed to assess classification performance regardless of rearing system. For breed discrimination (Krškopolje vs. modern breeds), five principal components were used ($\alpha = 0.05$), with a broader set of variables excluded, including isotopic parameters and multiple elemental variables (e.g., Na, Al, P, Ca, Cr, Mn, Co, Cu, Zn, As, Sr, Mo, Cd, Sb, Ba, Hg, Tl, Pb; Table S3). The model achieved high sensitivity (96%) and specificity (89%), with a total efficiency (*TEFF*) of 92.1%. The final DD-SIMCA analysis included 68 reference samples (KP, $N = 35$; MB, $N = 33$) from conventional production systems. In addition, 19 commercially available pork samples labelled as Slovenian origin were evaluated to assess the practical applicability of the breed discrimination model. Fig. 5a shows the KP

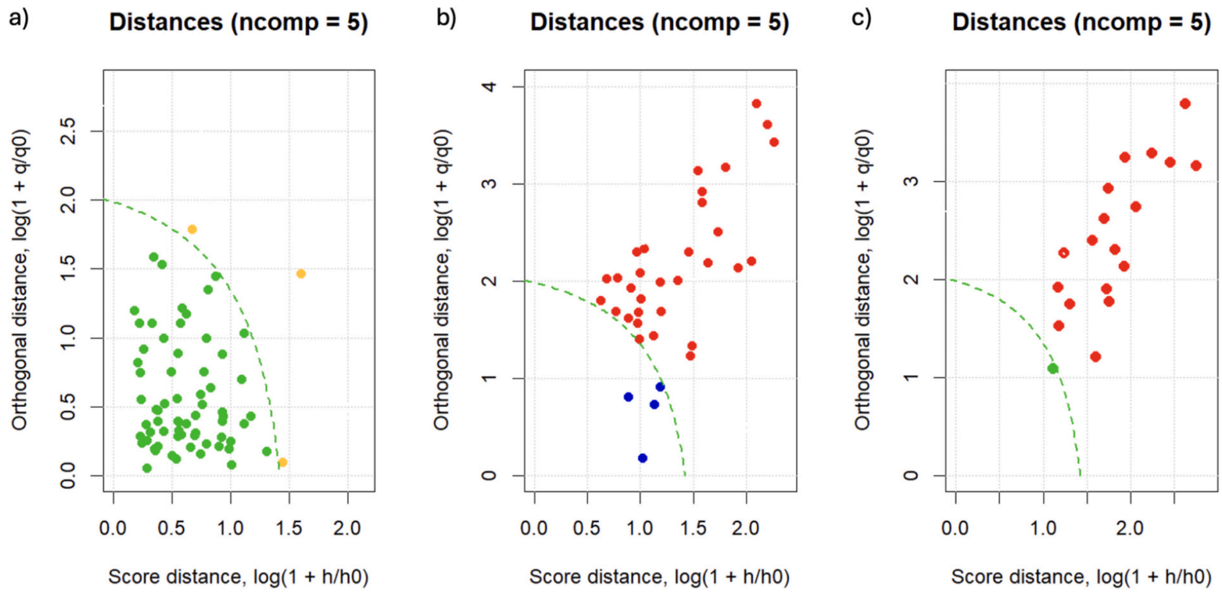

Fig. 5. DD-SIMCA classification model of (a) Krškopolje pig (KP) samples for construction of the model (n = 41), (b) modern breed samples (MB) (n = 42) for validation of the model, and (c) commercial samples from the market (modern breed) (n = 19) for additional validation of the model. The green dashed line represents the 100(1-α) % acceptance area, i.e., *the* decision boundary for whether the sample belongs to the target class at α = 0.05. The green points represent the correctly identified samples of the target class. The yellow points represent misclassified authentic KP samples. Red points in b) denote correctly classified MB samples, while blue points represent wrongly classified MB samples. In c), red points represent commercial MB samples that fell outside the model's acceptance region, indicating they are likely of non-KP breed (correctly classified), and green points located within the acceptance region represent commercial MB samples likely of KP breed (wrongly classified). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

samples used for model construction, with yellow dots indicating three samples that were misclassified. Fig. 5b shows authentic Slovenian modern breed samples used for model validation, with four misclassified samples indicated in blue. Fig. 5c presents the 19 commercially available pork samples labelled as Slovenian origin that were additionally evaluated using the DD-SIMCA breed discrimination model. Almost all

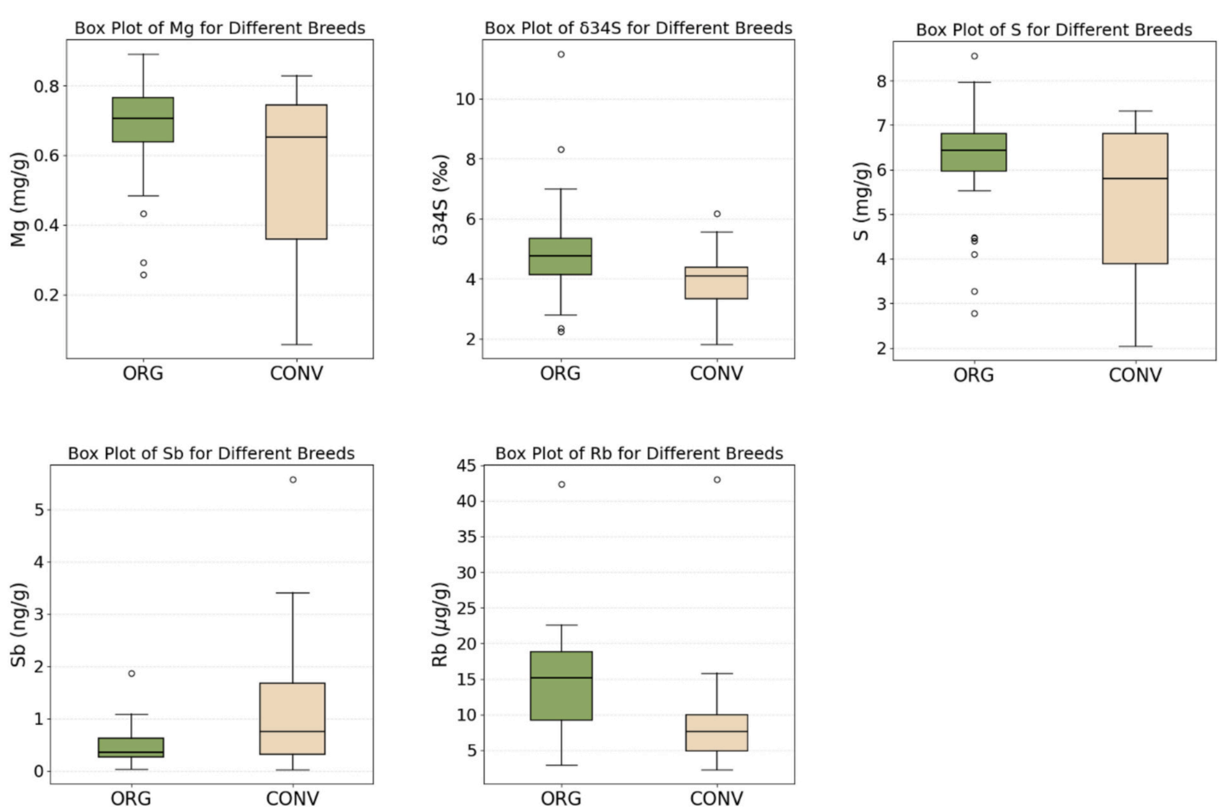

Fig. 6. Boxplots of statistically significant parameters (Mann-Whitney, P < 0.05) for differentiation between ORG (green) and CONV (beige) Krškopolje pig (KP) meat: δ^{34}S (‰), Mg, S and Sb (expressed in mg/g) and Sb (ng/g). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

commercial samples, except one (green dot), were correctly classified. In this case, the breed of all samples was known (modern breed), but their geographical origin is unreliable (samples are from the market). These samples thus provided additional validation of the model. Classification of commercially available KP samples was not performed, as no such samples were available on the market at the time of the study.

These results indicate that DD-SIMCA classification, based on a combination of stable isotope ratios and elemental composition, can be a practical approach for distinguishing KP and MB sample groups under the investigated production conditions. However, because breed and production-system effect could not be completely separated in the present study, further studies under control and comparable rearing conditions would be required to isolate breed-specidic effect.

3.3. Influence of rearing system (Krškopolje pig - KP) – organic vs conventional

To evaluate the influence of the rearing system, a Mann–Whitney test was applied to determine which measured variables significantly distinguished between organic (ORG) ($N = 37$) and conventional (CONV) (N = 35) production in Krškopolje pigs, where sufficient data were available to support statistical evaluation. The analysis identified δ^{34}S, Mg, Rb, and Sb as variables showing statistically significant differences ($P < 0.05$). The boxplots (Fig. 6) illustrate the distribution of these variables in Krškopolje pig (KP) samples from organic (ORG) and conventional (CONV) production systems.

In addition, an OPLS-DA model was applied to assess multivariate discrimination between the organic and conventional rearing systems. The model demonstrated partial separation between ORG and CONV samples, although the predictive performance was limited, as indicated by $R^2Y = 0.436$ and $Q^2 = 0.205$. The permutation tests ($n = 200$) yielded R^2 intercept of 0.19, and Q^2 intercept of −0.235. CV-ANOVA further confirmed the model's robustness, with highly significant *P*-values (< 0.0035).The overall prediction accuracy reached 80.6% (82.7% for CONV and 78.5% for ORG). Variables with VIP > 1 identified Mg, δ^{34}S, S, Sb, Rb, P, K, Cr, As, and Na as the most influential contributors to group separation. These results suggest that isotopic and elemental differences associated with production system exist, although the relatively low Q^2 value indicates limited predictive robustness of the model.

From the boxplots (Fig. 6), ORG samples generally exhibited higher Mg, δ^{34}S, δ^{15}N and Rb values, whereas CONV samples showed elevated Sb concentrations. Variations in these parameters may reflect differences in feed formulation, mineral supplementation practices, and production system characteristics between organic and conventional rearing systems. Feed used in conventional pig production is often composed of mixed feed components sourced from various regions (Perini, Camin, Sánchez Del Pulgar, & Piasentier, 2013), whereas organic production systems may rely more strongly on locally produced feed and impose greater restrictions on mineral additives use (Zhao et al., 2020). Previous studies have similarly demonstrated that combinations of stable isotopes and elemental profiles, including Ca, Mg, K, Na, Fe and δ^{15}N, can contribute to the differentiation of pork produced under conventional and organic feeding regimes (Zhao et al., 2020). The elevated Sb concentrations observed in conventionally produced samples may reflect differences in feed composition or environmental exposure associated with production systems. Elements such as Sb may originate from both natural and anthropogenic sources and can enter the food chain through soil, water, feed, or food-contact materials (Fernández-Arribas, Izquierdo, Bartrolí, Eljarrat, & Moreno, 2026). However, the specific source of Sb variability could not be conclusively determined in the present study. Analytical contamination and instrumental artefacts were minimised through standard quality control procedures during ICP-MS analysis.

δ^{15}N values are commonly used as indicators of farming practices and the type of nitrogen fertilisers applied to feed crops (Nie et al., 2020; Piasentier et al., 2003), with lower δ^{15}N values typically associated with the use of synthetic nitrogen fertilisers (Zhao et al., 2020). However, no significant differences were observed between organic and conventional KP meat, suggesting substantial overlap in feeding and management practices between the two production systems and highlighting the limited ability of δ^{15}N alone to discriminate between them.

4. Conclusions

This study demonstrated the potential of a combined stable isotope and elemental profiling approach for the authentication of Slovenian pork. Significant differences in isotopic and elemental composition were observed between the autochthonous Krškopolje pig and modern commercial breeds, reflecting differences in feeding practices, production systems, and environmental influences. Among the investigated variables, stable isotope ratios, particularly δ^{13}C, δ^{2}H, $\delta^{18}O_{wat}$, and δ^{34}S, together with selected trace elements such as Rb and Se, provided the most informative markers for characterising geographical and production-related variability.

The DD-SIMCA model developed using authentic Slovenian and non-Slovenian reference samples achieved a high classification performance, demonstrating the suitability of isotopic and elemental fingerprints for distinguishing pork consistent with the Slovenian reference production class. Application of the model to commercially available products further illustrated the potential of the approach for supporting traceability verification under market conditions. However, classification results should be interpreted as an assessment of consistency with the established Slovenian reference class rather than as definitive evidence of legal origin status or labelling compliance.

The findings also highlight the importance of comprehensive and well-characterised reference databases. Although the present study provides a robust proof of concept, further expansion of the database to include additional production years, broader geographical coverage, and greater variability in production systems will be necessary to evaluate long-term robustness and support routine implementation. Future work should also integrate more detailed production metadata, including feeding practices and farm management information, to further improve model interpretability and authentication performance.

Overall, the results demonstrate that the integration of stable isotope and elemental analysis represents a promising complementary tool for pork origin authentication and traceability verification, contributing to increased transparency, consumer confidence, and protection of geographically differentiated food products.

CRediT authorship contribution statement

Katja Babič: Writing – original draft, Visualization, Software, Methodology, Investigation, Data curation, Conceptualization. **Lidija Strojnik:** Writing – review & editing, Visualization, Software, Investigation, Data curation. **Doris Potočnik:** Methodology, Data curation. **Marta Jagodic Hudobivnik:** Methodology, Formal analysis. **Darja Mazej:** Data curation. **Luana Bontempo:** Writing – review & editing, Methodology, Formal analysis, Data curation. **Andrej Kastelic:** Writing – review & editing, Project administration, Funding acquisition. **Nives Ogrinc:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

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

While preparing this work, the authors used ChatGPT for translation and grammar purposes. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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.meatsci.2026.110166.

Data availability

Data will be made available on request.

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