

Authentication of beef from a Brazilian Cerrado terroir using elemental fingerprints and machine learning

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Abstract

Brazil is the world's largest producer and exporter of beef, with production distributed across all six biomes, which present distinct environmental and production characteristics. These particularities may contribute to the identity of beef. This study evaluated the potential of elemental profile to characterize and authenticate beef originating from the Cerrado terroir. Neutron activation analysis (NAA) and inductively coupled plasma mass spectrometry (ICP-MS) techniques were employed. The mass fractions of the chemical elements were associated with machine learning algorithms, resulting in classification models with 100% accuracy for the authentication of Cerrado terroir beef.

Introduction

Brazil has six distinct biomes: the Amazon, Caatinga, Cerrado, Atlantic Forest, Pampa, and Pantanal. Beef cattle production is practiced across all these biomes under diverse environmental and management conditions, supporting the use of a wide range of breeds, including Angus, Caracu, Devon, Gir, Guzerá, Hereford, Nelore, Senepol, Shorthorn, and Wagyu [1]. The country has the largest commercial cattle herd in the world, with more than 195.5 million head of cattle [2]. Among the country's biomes, the Cerrado—the second-largest biome in Brazil—stands out as the primary cattle-producing region, accounting for approximately 34% of the national herd [3]. The diversity of environmental conditions and production systems across the Brazilian territory provides a favorable context for the development of products with distinctive characteristics associated with their geographical origin [4].

Terroir is understood as the combination of physical characteristics, such as soil, climate, and vegetation, and anthropogenic factors, such as management practices, people, and culture [5]. In beef production, these conditions may directly influence the absorption, deposition, and metabolism of chemical elements in animal tissues, resulting in a characteristic elemental profile [6]. Therefore, the elemental profile may serve as an intrinsic signature of the product, supporting the use of multi-elemental approaches for the authentication and traceability of the geographical origin of beef [7]

The Cerrado presents considerable potential for the consolidation of distinct *terroirs* due to the extensive environmental and edaphoclimatic heterogeneity of this biome, encompassing differences in climatic conditions, soil types, nutrient availability, and vegetation composition [8]. Beef production within specific regions of the Cerrado may contribute to the incorporation of distinctive chemical characteristics into animal tissues, resulting in an elemental profile associated with cattle raised under the particular environmental conditions of each region [9].

Globally, some high-value meats are recognized for their association with territories and production systems with unique characteristics, in which environmental conditions, production practices, and local characteristics contribute to product differentiation. Examples of this association include meats from Prince Edward Island (Canada), Hokkaido Tokachi Farm (Japan), Grass Fed Irish Food (Ireland), Charolais de Bourgogne (France), and Carne Certificada Pampa (Brazil). These examples demonstrate that the interaction between territorial characteristics and production systems constitutes an important

component of the identity and value of meat products. The characterization of attributes capable of reflecting the influence of the territory is relevant for understanding and differentiating meats associated with distinct geographical regions.

Different analytical approaches have been employed to discriminate geographical origin and authenticate meat products. Among these, DNA-based techniques stand out for tracing the genetic origin of animals, confirming product authenticity, and identifying species [10, 11]. The combined use of stable isotopes and multi-elemental profiles has been employed to determine geographical origin, diet, and production systems [12]. Elemental profiling has also been applied to discriminate the provenance of animal-derived products [13, 14], while isotopic ratios have been used for food authentication [15]. In addition, chemometric methods combined with spectroscopic and chromatographic techniques, such as Raman spectroscopy and gas chromatography–mass spectrometry (GC/MS), as well as other spectral approaches, have been used to characterize chemical profiles associated with the authenticity and provenance of meat products [16–18]. In this context, the determination of elemental profiles by neutron activation analysis (NAA) has demonstrated high metrological capability for discriminating beef products from different geographical regions [1, 19]. More recently, blockchain-based systems have been incorporated into authentication processes, enabling the secure and transparent recording of information throughout the production chain [20–22].

Despite these advances, studies focused on the authentication of beef produced within specific *terroirs* remain scarce in Brazil, highlighting an important knowledge gap. Considering that the environmental, production, and cultural characteristics associated with *terroir* may influence the incorporation of chemical elements into animal tissues, this study characterized the elemental profile of beef samples from a *terroir* within the Cerrado biome using NAA and inductively coupled plasma mass spectrometry (ICP-MS), aiming to evaluate the potential of the resulting data for developing discrimination models to authenticate beef produced within this *terroir*.

Materials and methods

The study was conducted at Beef Passion, Nhandeara, São Paulo, Brazil, whose production system is characterized as a *terroir* in the Cerrado. This classification considers the integration between local environmental conditions and the specific characteristics of the production system, including management, nutrition, genetics, and traceability, which are maintained in an integrated manner throughout the production chain. The interaction among these factors creates the ideal conditions to confer unique characteristics to the product. The company operates under a vertical production system, responsible for all stages, from breeding to slaughter and commercialization, ensures complete traceability of the animals and is considered a *terroir* for beef production in Brazil [23]. Samples (n = 59) were collected at “Olhos d’Água Indústria e Comércio de Carnes Ltda.” slaughterhouse in the municipality of Ipuã, São Paulo, Brazil, where all samples from the Beef Passion company were collected. The samples originated from animals belonging to two different genetic crossbreeding groups: $\frac{1}{2}$ Angus – $\frac{1}{2}$ Nelore (AN) and $\frac{1}{4}$ Angus – $\frac{1}{4}$ Nelore – $\frac{1}{2}$ Wagyu (ANW).

The animals from the Cerrado biome that were not part of the *terroir* were collected in partnership with Fazenda Turbilhão, located in the municipality of Estrela d'Oeste, São Paulo, Brazil. The animals slaughtered were 100% traceable through the Brazilian individual cattle identification system and originate from different locations within the Cerrado. Samples (n = 48) were collected from five municipalities in the Cerrado, as shown in Fig. 1: Itápolis (IT), São Paulo; Piracanjuba (PI), Goiás; Planura (PL), Minas Gerais; Ponta Porã (PP), Mato Grosso do Sul; and Rondonópolis (RN), Mato Grosso.

The cut selected for the study was the biceps femoris muscle (picanha), as it is considered a premium cut with high added value and is widely available in Brazil. The external fat layer was removed from the samples, and the muscle was portioned into 300-g portions and cut into cubes using a titanium knife. The samples were then frozen and subsequently freeze-dried for 6 days. After freeze-drying, the samples were ground using an MM 500 nano mixer mill (Retsch), equipped with an aluminum oxide jar and grinding balls.

For NAA, 300 mg of sample was placed into high-density polyethylene capsules from Posthumus Plastics (Beverwijk, Netherlands). The certified reference materials SRM1566b Oyster Tissue and SRM1577c Bovine Liver from the National Institute of Standards and Technology (NIST) were used for analytical quality control. Analytical blanks consisted of empty capsules. For neutron flux monitoring, 10 mg fragments of a Ni-Cr alloy with a previously established composition were placed between the capsules [24]. Sample irradiation was performed in the IEA-R1 nuclear research reactor of the Nuclear and Energy Research Institute of the National Nuclear Energy Commission (IPEN/CNEN), located in São Paulo, SP, Brazil, 200 km from Piracicaba, SP, where the Center for Nuclear Energy in Agriculture (CENA) is located. Irradiation was carried out for 4 h at a neutron flux of approximately $1.2 \times 10^{13} \text{ cm}^{-2} \text{ s}^{-1}$, followed by a cooling period of 48 to 72 h before transportation to the Radioisotope Laboratory (CENA). Induced radioactivity was measured by high-resolution gamma-ray spectrometry using ORTEC high-purity germanium (HPGe) detectors, with relative efficiencies of 50% and 55% for the 1332 keV photopeak of ^{60}Co [1]. The mass fractions of the chemical elements were calculated using the k_0 method with Quantu software [25].

For ICP-MS, digestion of the samples, analytical blanks, and certified reference materials RM8414 Bovine Muscle, SRM1577c Bovine Liver, and SRM1566b Oyster Tissue from the National Institute of Standards and Technology (NIST) was performed. A 250 mg portion of freeze-dried and ground samples was placed in a polytetrafluoroethylene (PTFE) tube, together with 1 mL of hydrogen peroxide (H_2O_2) (30% m/m), 4 mL of nitric acid (HNO_3) (65% m/v), and 1 mL of Milli-Q IQ 7000 ultrapure water. The configuration used in the Milestone Ethos Up® microwave system was 35 min at 200°C and 1300 W, adapted from a previous work [26]. Subsequently, the digested samples were brought to a final volume of 50 mL and diluted to meet the maximum residual acid limit permitted by the equipment (3%). An inductively coupled plasma tandem mass spectrometer (ICP-MS/MS) with a triple quadrupole (Agilent Technologies 8900) was used. Standard solutions at concentrations of 1000 mg L⁻¹ from Sigma-Aldrich and Inorganic Ventures were used for each chemical element, comprising six different multi-element

standards for calibration. The spectrometer was operated at 1550 W power, with a plasma flow rate of 15 L/min and a nebulizer flow rate of 0.5 L/min. The internal standards used were ^{209}Bi , ^{72}Ge , and ^{103}Rh .

The determined mass fraction values of the chemical elements were evaluated by comparison with the certified values of the reference materials. For the study of the *terroir* and non-*terroir* groups, the following statistical tests were applied in RStudio: the Kruskal–Wallis test for ranking the variables based on the chi-square result; and the machine learning algorithms Classification and Regression Trees (CART), Multilayer Perceptron (MLP), Naïve Bayes (NB), Random Forest (RF), and Sequential Minimal Optimization (SMO), to assess the potential use of the elemental profile for sample discrimination. Samples from the Cerrado biome not associated with the *terroir* were grouped for comparison with the *terroir* samples, and the models were applied using the Weka software. The 10-fold cross-validation method was employed to separate the samples into test and training groups, considering the chi-square results to determine the importance ranking of the elements [27, 28]. *Terroir* discrimination was evaluated based on accuracy. Exploratory algorithms, such as hierarchical clustering (dendrogram), were used to assess the similarity among the municipalities of origin of the Cerrado samples, while PCA/Biplot was used for *terroir* discrimination.

Results and discussion

The elements Ba, Ca, Co, Cr, Cs, Cu, Fe, K, Mn, Mo, Na, Rb, Sr, and Zn were quantified. For the elements determined simultaneously by NAA and ICP-MS, good agreement was observed between the results, as demonstrated in Fig. 2. When applying the Pearson correlation coefficient (r), the highest correlation was observed for Rb ($r = 0.935$), followed by Cs ($r = 0.888$), Fe ($r = 0.869$), and K ($r = 0.862$), demonstrating the agreement between the two techniques [26].

When samples from municipalities located outside the *terroir* were applied to the CART, MLP, NB, RF, and SMO machine learning classification models, classification accuracies of only 33%, 31%, 31%, 35%, and 35%, respectively, were obtained (Table 1) to differentiate the origin of the samples among the five cities. These samples from the Cerrado biome originated from farms and producers with intermediate and similar levels of production systems and technical-scientific development, according to the IBGE [29]. Since the non-*terroir* Cerrado biome samples showed low discriminatory capacity among municipalities, the samples could be grouped for comparison with the *terroir*.

Table 1
Classification performance of machine learning models for discrimination among the five municipalities of origin within the Brazilian Cerrado, based on stratified 10-fold cross-validation

Model	Correctly classified	Incorrectly classified	Accuracy (%)	Kappa
CART	16	32	33.33	0.166
MLP	15	33	31.25	0.138
Naïve Bayes	15	33	31.25	0.137
Random Forest	17	31	35.42	0.192
SMO	17	31	35.42	0.187

Table 2 shows the median mass fractions and standard deviations of Ba, Ca, Co, Cr, Cs, Cu, Fe, K, Mn, Mo, Na, Rb, Sr, and Zn for the *terroir* samples from the Cerrado biome and the grouped non-*terroir* municipalities.

Table 2
Median and standard deviation (SD), expressed in mg/kg of dry mass, for the beef samples. P-values for the Kruskal-Wallis test comparing the two origins

Elements	<i>Terroir</i> (n = 59)		<i>Non-Terroir</i> (n = 48)		P valor
	Median	SD	Median	SD	
Ba	0.0688	0.220	0.181	0.0349	< 0.001
Ca	131	19	176	33	< 0.001
Co	0.00605	0.00760	0.0171	0.0113	< 0.001
Cr	0.111	0.0472	0.603	0.0454	< 0.001
Cs	0.128	0.0313	0.126	0.0688	0.726
Cu	2.67	0.529	2.90	0.683	0.118
Fe	62.8	8.70	63.5	7.71	0.194
K	9671	867	12604	1325	< 0.001
Mn	0.333	0.0714	0.250	0.0443	< 0.001
Mo	0.0300	0.00610	0.0357	0.00589	< 0.001
Na	1738	142	1920	225	< 0.001
Rb	22.7	4.1	9.43	2.9	< 0.001
Sr	0.206	0.126	0.297	0.0722	< 0.001
Zn	117	20.9	121	22.1	0.180

Based on this elemental profile, a study was conducted to explore the distribution of cuts from the *terroir* and cuts from other regions of the Cerrado biome outside the *terroir*. Figure 3 shows the principal component analysis used to differentiate the Beef Passion samples (red), accounting for 51.6% of the information (PC1 36.5% and PC2 15.1%).

The distribution of the samples in the multivariate space showed a clear separation between the two groups, mainly along PC1, with the Beef Passion samples concentrated on the negative side of this component. This pattern indicates that the differences in elemental profiles were sufficiently consistent to differentiate the two origins. In the loading plot, Rb and Mn showed vectors oriented toward the *terroir* sample cluster, indicating an association of these elements with the position of the samples in this group. In the opposite direction, Cr, K, Ca, Sr, Co, Mo, Na, Cu, Zn, Fe, Cs, and Ba showed vectors predominantly oriented toward the positive side of PC1, associated with the non-*terroir* samples. PC2,

responsible for 15.1% of the variability, contributed mainly to the distribution of samples within the groups, highlighting secondary differences in the elemental profile.

Figure 4 shows the hierarchical cluster analysis (HCA), based on Euclidean distance, which allowed the similarity among the six origins studied to be evaluated. The dendrogram revealed the existence of three main clusters. The NH samples were positioned separately in the dendrogram, showing greater separation from the others at the largest Euclidean distance observed in the study (9.0), demonstrating differences in their elemental profiles and confirming the separation observed by PCA. Among the remaining groups, PL and RN formed the most cohesive core and showed the greatest similarity (distance of 1.6), to which PP joined to form a first major cluster. In parallel, IT and PI grouped into a distinct second subgroup (3.8), establishing a clear separation among the groups, between the atypical group (NH), the PL/RN/PP cluster, and the IT/PI pair.

Before applying the machine learning models, the 14 determined elements were evaluated for their discriminatory power using the Kruskal–Wallis test, using the chi-square value to rank the variables. The ten elements with the highest chi-square values were selected for the construction of the models to differentiate the samples according to *terroir*. This step allowed the dimensionality of the dataset to be reduced, minimizing the contribution of less informative variables and facilitating model interpretation, while highlighting the elements with the greatest potential to reflect differences associated with the characteristics of the *terroir* in this study (Fig. 5).

The machine learning models showed consistent performance in discriminating Beef Passion (BP) and non-Beef Passion (NBP) samples, with 100% accuracy and a Kappa coefficient of 1.0 for Random Forest, CART, Multilayer Perceptron (MLP), Naïve Bayes, and SMO, using 10-fold cross-validation (Table 3). Random Forest achieved this performance by simultaneously considering ten chemical elements: Mn, Rb, Ba, Cr, Co, Sr, Mo, Na, Ca, and K. The other models also used the same ten elements but identified Cr and Rb as chemical predictor variables. The discriminative capacity observed in the tree-based models also highlights the relevance of specific elements. For Cr, CART established a cutoff point of 0.7, classifying samples below this value as BP and those greater than or equal to this threshold as NBP, with no classification errors (100% accuracy). Similarly, Rb showed high discriminative capacity, with 98% accuracy and a cutoff point of 16.49, as shown in Fig. 6. These results are particularly relevant because they indicate that the differentiation between the groups is associated with marked differences in the elemental profile of the samples, with Cr and Rb showing clear discriminative capacity. In the case of Cr, this interpretation is further corroborated by SMO, which used Cr in the construction of the linear classifier.

Table 3

Classification performance of the machine learning models used to discriminate terroir (BP) and non-terroir (NBP) beef samples based on their elemental profile

Model	BP correctly classified	NBP correctly classified	Misclassified	Accuracy	Kappa
CART	59/59	48/48	0	100%	1.00
MLP	59/59	48/48	0	100%	1.00
Naïve Bayes	59/59	48/48	0	100%	1.00
Random Forest	59/59	48/48	0	100%	1.00
SMO	59/59	48/48	0	100%	1.00

The results suggest that Cr and Rb may constitute potential elemental markers for differentiating BP and NBP samples. The hypothesis is that the relevance of these elements may be associated with differences in environmental and production conditions between the systems evaluated, since the availability of these elements in the environment and their incorporation by animals may be influenced by soil composition, vegetation, and feed [6]. Thus, the behavior of Rb and Cr may be related to geochemical differences between the systems. Rb showed a greater association with the *terroir* samples, whereas Cr was more closely related to the non-*terroir* samples, a pattern observed in both the principal component analysis (PCA) and the CART model. From a geochemical perspective, Rb may reflect differences in parent material and in weathering and leaching processes, whereas Cr has a greater association with the mineral fraction and may potentially be influenced by soil mineralogy and degree of weathering. Thus, the differences observed for these elements may contribute to the formation of distinct elemental profiles between the systems evaluated, reinforcing their potential as discriminant variables of animal origin [30].

The differentiation observed between the Beef Passion samples and the other Cerrado samples may be related to the combination of environmental and production factors that characterize the system studied. Although all samples originate from the Cerrado biome, the heterogeneity of the biome, edaphoclimatic conditions, vegetation composition, management, feeding, and genetic characteristics of the animals may contribute to different patterns of chemical element incorporation into the tissues. Therefore, the association between local environmental characteristics and the specific production system of Beef Passion may contribute to the formation of intrinsic characteristics in beef.

Conclusion

The elemental profile obtained for the beef samples from the Cerrado *terroir* and the other samples from the biome comprised a total of 14 elements: Ba, Ca, Co, Cr, Cs, Cu, Fe, K, Mn, Mo, Na, Rb, Sr, and Zn. The machine learning models applied to the beef samples from the Cerrado biome and the *terroir* system demonstrated potential for authentication, with 100% accuracy for the evaluated dataset, using a group

of 10 elements, with emphasis on Cr and Rb. These results indicate that beef originating from the Beef Passion *terroir* presents a characteristic elemental profile capable of enabling its discrimination.

Declarations

Author Contribution

T.K. wrote the original draft of the manuscript and contributed to methodology, visualization, investigation, formal analysis, data curation, and conceptualization. E.F. contributed to conceptualization, review and editing, visualization, supervision, project administration, and funding acquisition. Y.M. contributed to formal analysis, investigation, software, data curation, and visualization. M.B. contributed to software, formal analysis, and investigation. C.G. contributed to investigation and resources. All authors reviewed the manuscript.

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Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on request.

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Figures

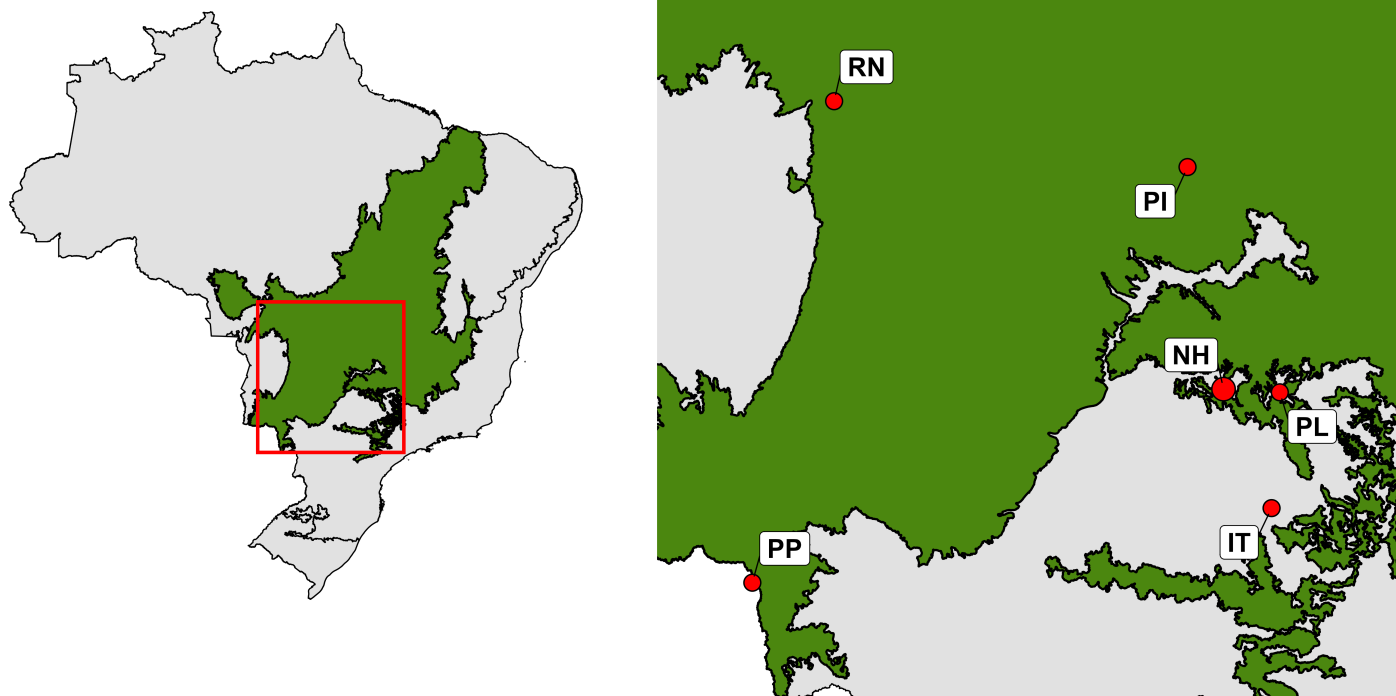


Figure 1

Sampling locations of beef in the Cerrado biome (green): IT: Itápolis; NH: Nhandeara (*Terroir*); PI: Piracanjuba; PL: Planura; PP: Ponta Porã; RN: Rondonópolis.

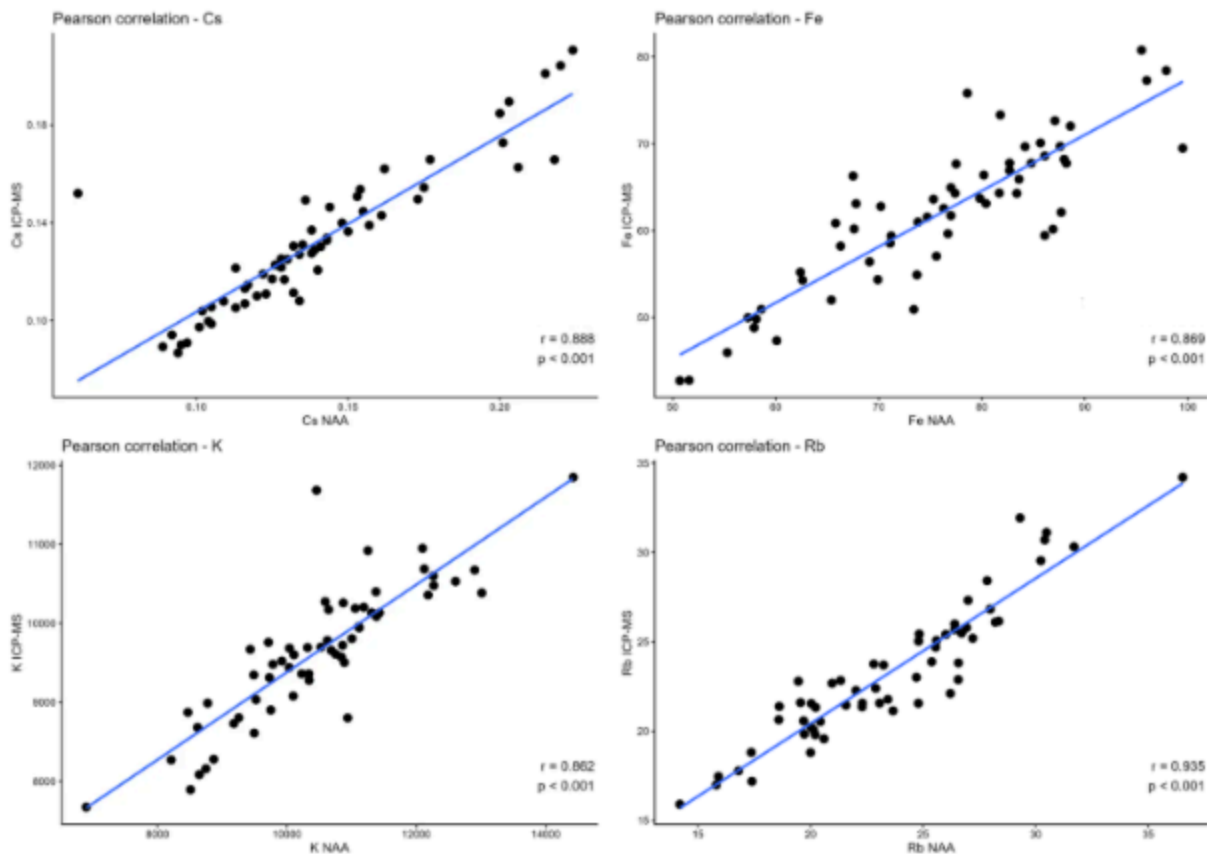


Figure 2

Correlation of the results obtained by NAA and ICP-MS for beef samples from terroir and non-terroir sources for Cs, Fe, K, and Rb, Pearson correlation coefficient (r), and p-value

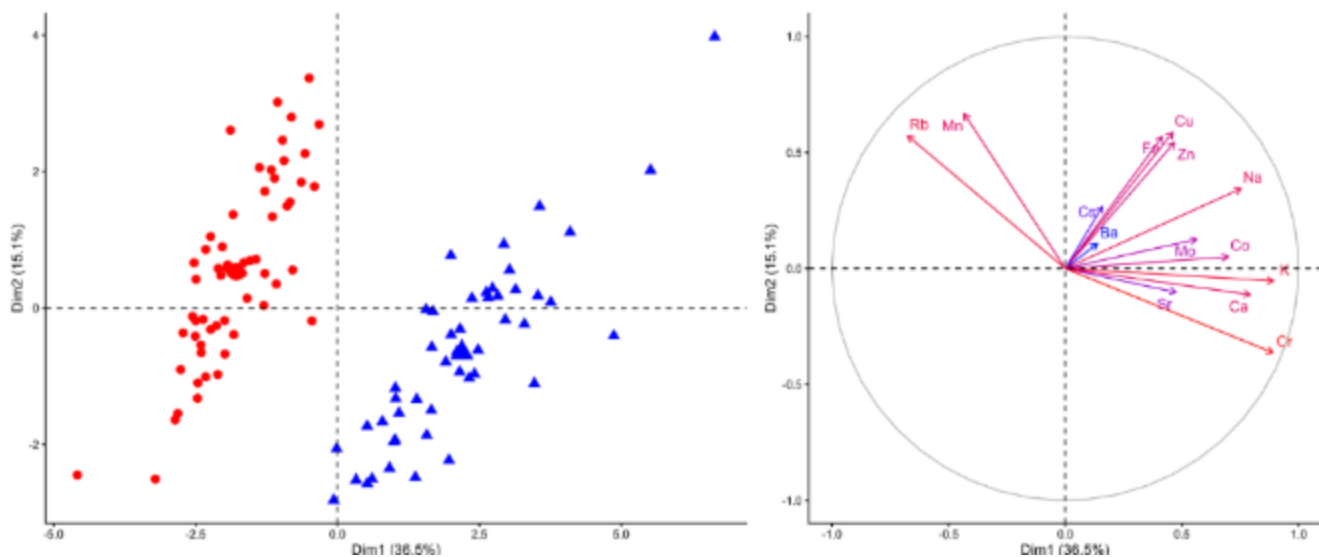


Figure 3

Principal Component Analysis for discriminating samples from the *terroir* (n = 59) (red) and non-*terroir* (n = 48) (blue), using the 14 determined elements and explaining 51.6% of the information (PC1 36.5% and

PC2 15.1%).

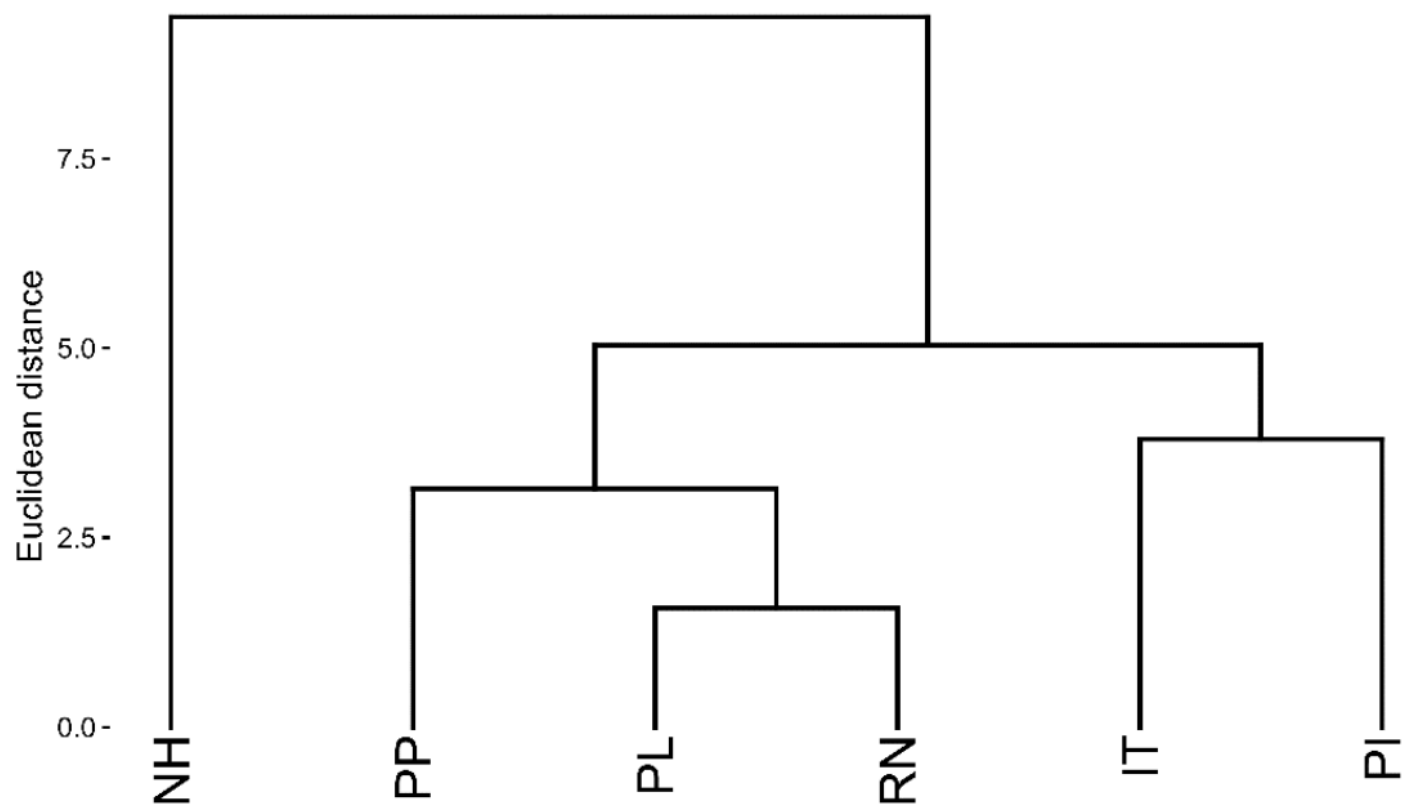


Figure 4

Dendrogram of the municipalities where beef samples were collected, generated from the elemental profile. IT: Itápolis; PI: Piracanjuba; PL: Planura; PP: Ponta Porã; RN: Rondonópolis; NH: Nhandeara, SP.

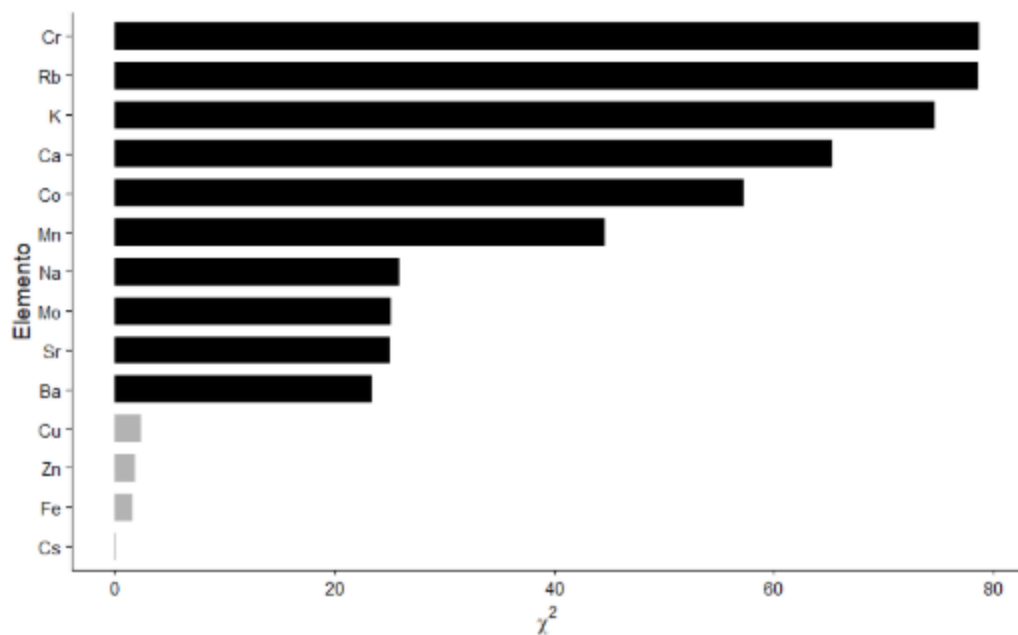


Figure 5

Ranking of discriminating elements for *terroir* and non-*terroir* beef samples based on chi-square values; elements used in ML for discrimination are shown in black.

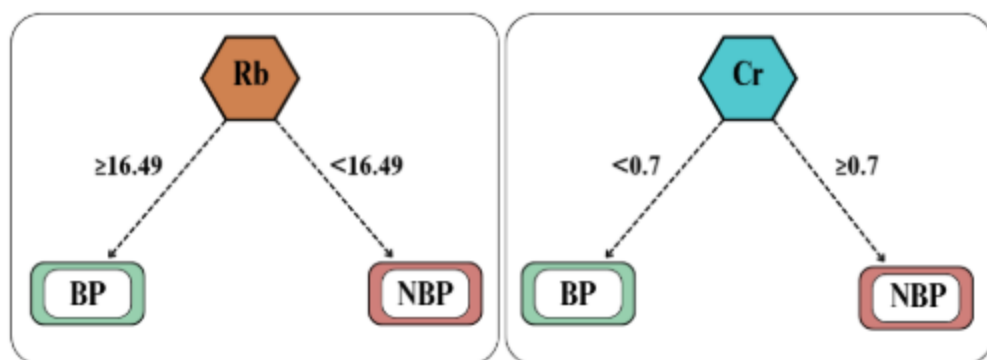


Figure 6

Decision tree generated by the CART model for discriminating *terroir* (BP) and non-*terroir* (NBP) samples using Rb and Cr concentrations as markers.