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Impact of mineral and organic fertilisation practices on elemental authenticity signature on apple Royal Gala from protected geographical indication (PGI) "Maçã de Alcobaça"

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A R T I C L E I N F O

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A B S T R A C T

Market demand, climate change and soil degradation force producers to maintain the productivity and quality of high-market-value products, such as Protected Geographical Indication (PGI) fruits. "Maçã de Alcobaça" apples produced in the central-western part of Portugal, namely the variety Royal Gala, one of the PGI varieties with higher demand, are among those with higher requirements in terms of fertilization to maintain the high productivity demanded by the market. In the present work, three different soil NPK fertilization schemes were applied to experimental orchards within the PGI area (1 x mineral NPK proposed for integrated production, an intermediate strategy that included organic granular amendment and 2 x mineral NPK), and the elemental profiles of the apple pulps were analysed and compared. Some mineral elements improved their concentration in the apple pulps with fertilization due to interactions of these elements with the fertilizer components (namely, nitrogen, potassium and phosphorus) or to potential changes in the bioavailability of the elements in the soil due to fertilization application. From a nutritional perspective, enhancing the mineral profile of apple pulps can be achieved by applying 1 x NPK fertilization. Consuming an average of 2 fruits daily (160 g each) would then help meet a higher percentage of the daily requirements for most essential elements crucial for human nutrition. Also, noteworthy to mention, that none of the tested fertilization practices led to a reduction in the nutritional quality of the fruits analysed when compared to the 1 x NPK condition. The present work also had the objective of evaluating if these fertilization practices and the mineral changes induced would have implications for the PGI authenticity elemental signatures previously developed. Using Partial Least Squares-Discriminant Analysis (PLS-DA) models calibrated with PGI and non-PGI (from North Portugal and Italy) samples and feeding these models with elemental profile data of fruits collected from fertilized orchards as blind samples, it was possible to observe that all samples from the fertilization trials were correctly classified as PGI samples. This reinforces the edaphic characteristics of the cultivation area's prevalent role over the effect of fertilization practices or physiological trait changes, in shaping the elemental signature of the fruits. This was found to be mostly due to the high influence of geologically linked elements (such as Rb, Pb and Y) in the discrimination of the sample provenance. This allows us to confirm the suitability of the elemental traceability models for "Maçã de Alcobaça" PGI authenticity validation, ensuring its provenance and nutritional characteristics to the consumers and maintaining its market value even if fertilization practices are applied to fight less favourable cultivation conditions.

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1. Introduction

The European Union (EU) confers the status of Protected Geographical Indication (PGI) to approximately 1257 food products, out of a total of 3416 protected products, with approximately 28 % of all PGI products being fruits, vegetables, and cereals, including more than 20 apple varieties (European Union., 2022). Protection status conferred on food products ensure their specific characteristics and quality, including nutritional value, are therefore highly appealing labels to the consumers. Apples from Alcobaça, Portugal, labelled "Maçã de Alcobaça" PGI, have historical roots dating back to the Middle Ages. Cultivated in regions like the West Coast and Alcobaça, their unique qualities thrive in diverse soil and climate conditions (Almeida et al., 2017). The European Union established in 2015 that the "Maçã de Alcobaça" PGI encompasses the cultivars Casa Nova, Golden Delicious, Red Delicious, Gala, Fuji, Granny Smith, Jonagold, Reineta and Pink produced in the defined geographically delimited area and characterized by elevated consistency and crispness, a high percentage of sugar and high acidity, which gives them a bittersweet taste and intense aroma (detailed information can be found at https://www.maca.pt) (European Commission, 2015).

The PGI label has boosted fruit production, now at 50,000 tons yearly, valued at 0.75 €/kg, generating 37.5 M€ annually. In contrast, non-PGI apples, selling at 0.65 €/kg, yield 32.5 M€/y (DGADR, 2016) (DGADR, 2016). In high-value markets like PGI products, food fraud is a growing concern. Non-PGI fruits are sometimes mislabelled to inflate their value, flooding the market with fraudulent products and impacting the market value of PGI goods. To address this, the EU has mandated member states to enhance food traceability and combat mislabelling (European Commission, 2002). The increasing obligations in terms of food security led to the application of geographical origin authentication procedures, allowing consumers to unambiguously have trustworthy information on the food product's origin (Leal et al., 2015).

"Maçã de Alcobaça" PGI production is bound to strict cultivation practices, including fertilization treatments allowed and limits imposed (DGADR, 2012). A previous work (Duarte et al., 2023b) has shown that the apple pulp elemental profiles can be efficiently used to discriminate the geographical origin of the apples and certify their PGI status. However, fertilization practices, namely the application of NPK and/or organic amendments, were already found to alter the fruit elemental composition of macronutrients. For example, high doses of annual soil applications of NPK fertilizers, whether organic or mineral, were found to affect the macronutrient composition of 'Daiane' apples (Hahn et al., 2018), whilst long-term annual surface fertilization with N and K was also found to impact macronutrients of 'Fuji' apples (Nava and Dechen, 2009). Previous works also found that rootstocks and K and N fertilizers can affect the mineral composition of apple fruit and leaf (Fallahi et al., 2021; Sotiropoulos, 2008). Conversely, increased N fertilization did not alter the content of macronutrients on fruits of the cultivar 'Jonagored', even when compared with apples from unfertilized trees (Kowalczyk et al., 2016), nor in apples from orchards of 'Gala' fertilized with different NPK levels and types (Mota et al., 2022). Therefore, it becomes of utmost importance to ensure that the nutritional profile of macro and micronutrients of the apples produced from orchards under different fertilization schemes is maintained as well as its elemental fingerprint, ensuring an efficient authentication of its "Maçã de Alcobaça" PGI denomination.

The environmental conditions (namely climatic and soil characteristics) under which plants are cultivated have the utmost effect on the multi-elemental profile of the plants or fruit products. Thus, these biochemical profiles have been extensively used as tools to validate the geographical origin of foodstuffs, covering a variety of food products, namely edible seaweeds (Duarte et al., 2023a), grains and cereals (Cozzolino, 2014; Duarte et al., 2022b) and even fruits and vegetables (Duarte et al., 2023b; Francois et al., 2020). Although several elemental analysis techniques are available (such as Inductively Coupled Plasma (ICP) spectroscopy and its coupled variations or Flame Atomic Absorption Spectrometry (FAAS)), the untargeted nature of total X-ray fluorescence (TXRF) spectroscopy has been pointed out as a high-throughput analytical tool to efficiently analyse food products' elemental composition and provide the necessary large datasets for chemometric approaches to trace the product's geographical origin while simultaneously providing precise food nutritional values (Kelly et al., 2005; Lim et al., 2021; Rajapaksha et al., 2017). Additionally, this is a low-cost technique when compared to other available analytical techniques, requiring minimal sample preparation, being fast and sensitive, and allowing multi-elemental quantitative profiling (Feng et al., 2021).

Having these factors in mind, it becomes important to evaluate the effects of different fertilization treatments on the nutritional value in terms of mineral nutrients of the most sold "Maçã de Alcobaça" cultivars, the Royal Gala, as well as to verify if the changes induced by fertilization practices affect the apple pulp elemental signature or not and if the PGI status can still be authenticated using previously developed chemometric models for the "Maçã de Alcobaça" PGI label. For this, two mineral fertilization practices and one mineral fertilization application supplemented with an organic amendment were tested in "Maçã de Alcobaça" PGI Royal Gala orchards, and the elemental profiles of the fruit pulp were analysed in terms of mineral nutrient valuation, but also tested using previously developed models to evaluate the maintenance or not of the PGI elemental signature under different fertilization schemes.

2. Materials and methods

2.1. Fertilization treatment and sample collection

The fertilization trial occurred at the National Institute of Agriculture and Veterinary (INIAV) experimental Royal Gala orchards, located within the "Maçã de Alcobaça" PGI geographical area. The PGI area is characterized by subcoral limestone and fine and clayey stoneware and is located in a transitional area between a mountain system and the Atlantic Ocean, with temperate-oceanic microclimatic conditions resulting from the humid Atlantic air, a mean annual air temperature of 15 °C and moderate annual rainfall (APMA, 2014). For the present study, three fertilization treatments were tested:

i) 1 x NPK Fertilizer (common practice in "Maçã de Alcobaça" PGI geographical area): Application of 200 kg/ha of 14:7:14 (N:P:K) (plus 14% CaO) (Nutricomplex Plus, Tradecorp, Rovensa, Portugal) and reinforcement after one and half months with 150 kg/ha of 15–5–30 (N:P:K) (plus 11.5 % SO_3, 2 % MgO, 0.02 % B, 0.06 % Fe, 0.04 % Mn, 0.02 % Zn, 0.01 %Cu) (Nutricomplex, Tradecorp, Rovensa, Portugal).
ii) 2 x NPK Fertilizer: Application of 400 kg/ha of 14:7:14 (N:P:K) (plus 14 % CaO) (Nutricomplex Plus, Tradecorp, Rovensa, Portugal) and reinforcement after one and half months with 300 kg/ha of 15–5–30 (N:P:K) (plus 11.5 % SO_3, 2 % MgO, 0.02 % B, 0.06 % Fe, 0.04 % Mn, 0.02 % Zn, 0.01 %Cu) (Nutricomplex, Tradecorp, Rovensa, Portugal).
iii) 1 x NPK Fertilizer + Organic Matter (OM): Application of 340 kg/ha 14–7–17 (N:P:K) (plus 22.5 % SO_3, 2 % MgO, 0.02 % B, 0.01 % Zn) (Entec Nitrofoska, Eurochem, Portugal) supplemented with 250 kg/ha granulated manure 4–3–2.5 (N:P:K) plus 65 % OM (Orgevit, Ecoveg, Portugal).

All fruit samples were collected at the end of the maturation season (Summer 2022) and brought back to the laboratory for further processing. Eight fruits were collected randomly from a pool of 15 trees per treatment. Apple pulp samples were collected using stainless steel cork cutter cylinders. The endocarp and exocarp were discarded from the collected cylinder. Mesocarp samples were stored at −80 °C and freeze-dried for 48 h at −50 °C (Telstar laboratory freeze dryer, Cryodos-45).

2.2. Sample processing and elemental analysis

All labware used for elemental analysis was decontaminated in acid baths for 48 h before use. Freeze-dried samples (10 independent replicates per class) were mineralized with HNO_3 (Nitric acid) in Teflon reactors, following a microwave digestion process (Multiwave GO Plus, Anton Paar GmbH, Graz, Austria) according to the EPA 3052 method (Environmental Protection Agency EPA., 1996). Briefly, the temperature profile is specified to permit specific reactions and incorporates reaching 180 ± 5 °C in approximately less than 5.5 min and remaining at 180 ± 5 °C for 9.5 min for the completion of specific reactions (Environmental Protection Agency EPA., 1996). After cooling, an internal standard (Gallium, final concentration 1 mg/L) was added to each sample, and 5 μL of each sample was then applied to a siliconized quartz disk (Bruker Nano Analytics, Germany) and dried. Elemental concentrations (Br, Ca, Cl, Cr, Cu, Fe, K, Mn, Na, Ni, P, Rb, S, Sr, Ti, V and Zn) were determined by total reflection. Elemental concentrations were determined by X-ray fluorescence spectroscopy (TXRF, S2 PICOFOX, Bruker Nano GmbH, Germany) considering the fluorescence values from the individual sample application of the internal standard (Duarte, et al., 2022a, 2022b).

Instrumental recalibration (gain correction, sensitivity analysis and multi-elemental standards) and analytical blanks were used for quality control, according to the manufacturer instructions and using Arsenic and Nickel standard discs (Bruker Nano GmbH, Germany) for calibration. Blanks with only HNO_3 were used to assess potential contaminations from the extraction process. Multi-elemental disc samples provided by the manufacturer were used to ensure the detection of a wide array of representative elements along the energetic segment surveyed (Bruker Nano GmbH, Germany). Extraction efficiency was confirmed through the analysis of International certified reference materials (ERM-CD281 Ryegrass, Table 1). Most of the certified elements presented an elevated extraction efficiency (>95%), ensuring that the applied extraction method has a very good recovery and is appropriate for this type of sample (Table 1).

2.3. Nutritional analysis

For the Daily Recommended Value (DRV) relative percentage, the average ingestion of 2 pieces of fruit per day (160 g each) was considered, following the World Health Organization (WHO) recommendations (World Health Organization, 2014). Dietary reference values were attained from the European Food and Safety Authority (EFSA). Adequate Intake (AI) or Average Requirement (AR) values were used depending on the availability of each parameter. Average values for adult men and women (non-pregnant) were considered for each element AI or AR (European Food and Safety Authority EFSA., 2019) (Table 2).

Table 1
Ryegrass (ERM-CD281) certified and analysed elemental values, uncertainty (mg/kg) and calculated extraction efficiency (average ± standard deviation, $N = 5$).

Element	Certified Value	Uncertainty	Measured Value	Extraction Efficiency (%)
Cr	0.73	0.22	0.67 ± 0.10	91.3 ± 5.3
Mn	4.88	0.24	3.35 ± 0.10	68.6 ± 1.9
Fe	161.0	8.0	198.03 ± 0.67	123.0 ± 0.4
Ni	0.69	0.15	0.78 ± 0.05	113.1 ± 5.8
Cu	5.98	0.27	7.10 ± 0.07	118,8 ± 1.0
Zn	71.0	4.0	73.29 ± 0.28	103.2 ± 0.4
As	6.7	0.4	7.25 ± 0.07	108.2 ± 0.9
Se	1.62	0.12	1.51 ± 0.03	93.3 ± 1.9
Rb	2.46	0.16	2.45 ± 0.05	99.6 ± 1.9
Sr	19.0	0.0	18.55 ± 0.34	97.6 ± 1.8
Cd	0.336	0.025	0.32 ± 0.02	96.6 ± 4.5
Pb	2.18	0.18	2.47 ± 0.05	113.3 ± 2.1

Table 2
Average Daily Recommended Value (DRV) for adult men and women (non-pregnant) of the mineral nutrients considered, according to the European Food and Safety Authority (EFSA, 2019).

Element	Daily Recommended Value (mg/day)
Ca	800
Cl	3100
Cu	1.45
Fe	6.0
Mn	3.0
Na	2000
Zn	9.16

2.4. Statistical analysis

Non-parametric Kruskal–Wallis with Bonferroni post hoc tests were used for pairwise comparisons between the elemental concentrations of the samples collected at the different geographical areas and were performed using the *agricolae* package (De Mendiburu and Simon, 2015). Boxplots with probability density of the data at different values smoothed by a kernel density estimator were computed and plotted along with a density plot using the *ggplot2* package (Wickham, 2009).

For the chemometric approach, a Partial Least-Squares Discriminant Analysis (PLS-DA) methodology was used, and a variable selection method was implemented, specifically variable importance in projection (VIP) of PLS-DA. Both analyses were performed using the *DiscriMiner* package (Sanchez, 2013) in R-Studio Version 1.4.1717 (R Core Team., 2021). As a training set a previously published dataset (Duarte et al., 2023b) consisting of PGI Royal Gala apple pulp elemental profiles, from orchards originating from the National Institute of Agriculture and Veterinary (INIAV) core collection located within the "Maçã de Alcobaça" PGI area (training set of 60 samples, approximately 71 % of the whole dataset), as well as Royal Gala apple fruits harvested from orchards located in the north of Portugal Royal Gala production sites (non-PGI PT) and from the Italian production region of Trentino-Alto Adige/Südtirol, in the country's far north (non-PGI IT). The number of PLS-DA classes was set to 2 (k=3–1, taking into consideration the three classes of samples used in the training model) for the PLS-DA models. Component number selection was validated by analysing parameters such as the Receiver Operating Characteristic (ROC) Area Under the Curve (AUC), goodness of fit (R^2), and goodness of prediction (Q^2) values. The statistical significance of the AUC parameter was evaluated using a Wilcox test and the component selection using ROC-AUC was performed using the *MixOmics* package (Rohart et al., 2017) in R-Studio Version 1.4.1717. After ensuring a correct number of components and high AUC values, model accuracy variable components coordinates were calculated using the *DiscriMiner* package (Sanchez, 2013) in R-Studio Version 1.4.1717. The Variable Importance in Projection (VIP) score was calculated using the *DiscriMiner* package (Sanchez, 2013) in R-Studio Version 1.4.1717. Having the training model produced, a test phase was conducted where the samples from the tested fertilization treatments were supplied to the trained model as blind (non-labelled) samples (test set of 25 samples, approximately 29 % of the whole dataset), and its allocation into the trained groups was evaluated as well as the test model accuracy as abovementioned.

3. Results

3.1. Nutritional profile under different fertilisation schemes

No significant changes were detected in the apple pulp Br, Cl, Cr, Na, S, Sr, Ti and V concentrations (Fig. 1A, C, D, I, M, N, O and P respectively) upon applying the different fertilization schemes tested. Pulp calcium concentration (Fig. 1B) was significantly increased in the apples collected from trees fertilized with 1 x NPK treatment. Copper concentration in the apples under 1 x NPK fertilization was found to be higher

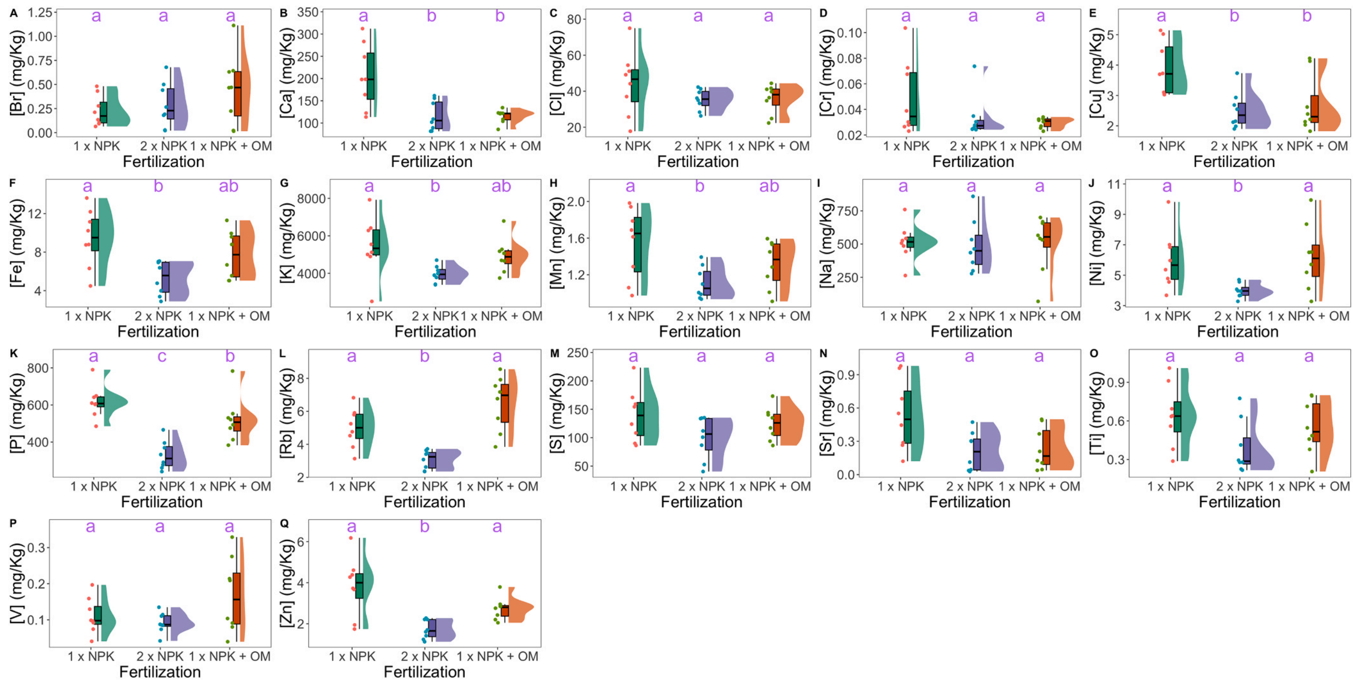

Fig. 1. Mineral element concentration in the apple pulp samples collected from trees subjected to the different tested fertilization schemes (N=8 per treatment, letters denote significant differences at $p < 0.05$).

than those verified in the remaining tests fertilization schemes (Fig. 1E). Regarding apple Fe, K, Mn, Ni, Rb and Zn, mesocarp concentrations were found to be reduced in the fruits harvested from the trees fertilized with 2 x NPK (Fig. 1F, G, H, J, L and Q respectively). Phosphorous pulp concentration showed lower values in the fruits from trees supplemented with 2 x NPK and 1 x NPK with organic matter addition, being more pronounced in the trees lacking organic supplementation (Fig. 1K).

When observing the apple pulp element's Daily Recommended Value (DRV) percentage (Table 3), several differences arise from the different fertilization schemes. Calcium DRV fulfilment percentage was significantly higher in the fruits collected from 1 x NPK fertilized trees when compared to the remaining treatments. On the other hand, no significant differences were found in the chlorine and sodium DRV relative values of the analysed fruits. Fruits from plants treated with 2 x NPK and 1 x NPK with organic matter had notably lower Cu DRV fulfilment percentages. Additionally, iron and Mn DRV were significantly lower in fruits from trees under 2 x NPK fertilization. The ingestion of the same apple fruit quantity resulted in a significantly lower Zn DRV fulfilment if the fruits from the trees fertilized with 2 x NPK were considered.

3.2. Fertilisation practices impact on the PGI traceability signature

Using elemental signatures from the Royal Gala apples produced within the "Maçã de Alcobaça" PGI area and outside (both in Portugal and Italy), it was possible to build a chemometric traceability model that efficiently classified the provenance of the apple pulp samples (Fig. 2). This was used as a training set for application to the samples originating from orchards with different fertilization practices, with a 100 % classification accuracy. When applying the trained model to a blind dataset of apple pulp samples from trees under various fertilization treatments, all test samples were consistently classified within the PGI category.

Analysing the model biplot with PLS-DA components as dimensions revealed that all fertilization treatment samples overlapped with the "Maçã de Alcobaça" PGI samples area (Fig. 3A), emphasizing their similarity as shown in the confusion matrices between PGI and fertilized samples. Assessing the significance of each element for discrimination and classification accuracy, numerous elements exhibited variable importance in projection (VIP) values above 1, indicating their relevance in explaining model performance (Fig. 3B). Notably, Rb emerged as the most influential discriminator, trailed closely by Pb and Mn, which displayed similar VIP scores. Among the most relevant elements driving the model performance were Na, Y, Cl and Br. Pb and Y were absent in samples from various fertilization treatments but were detected in non-PGI samples, hence flagged as significant elements by the model.

Table 3
Element Daily Recommended Value (DRV) percentage in the apple pulp samples collected from trees subjected to the different tested fertilization schemes (average ± standard deviation, N=8 per treatment, letters denote significant differences at $p < 0.05$).

Element	1 x NPK	2 x NPK	1 x NPK + OM
Ca	8.20 ± 2.89^{a}	4.63 ± 1.33^{b}	4.60 ± 0.60^{b}
Cl	0.46 ± 0.18^{a}	0.36 ± 0.06^{a}	0.37 ± 0.08^{a}
Cu	86.13 ± 19.24^{a}	55.33 ± 13.53^{b}	59.51 ± 20.82^{b}
Fe	50.34 ± 16.09^{a}	28.27 ± 9.31^{b}	41.29 ± 13.13^{ab}
Mn	16.45 ± 4.15^{a}	11.85 ± 1.87^{b}	14.01 ± 2.77^{ab}
Na	8.22 ± 2.22^{a}	7.79 ± 3.15^{a}	8.09 ± 3.43^{a}
Zn	13.33 ± 5.04^{a}	6.02 ± 1.60^{b}	9.56 ± 1.88^{a}

4. Discussion

Ongoing climate change has challenged crop productivity, particularly fruit production. An increase in atmospheric CO_2 concentrations due to climate change can positively affect fruit production, but also increase the demand for nutrients and water by plants (Fischer et al., 2022, for revision). Khalsa et al. (2020) also concluded that under warmer conditions intensive N fertilizer use not only increased N cycling but also ameliorated the effects of warmer cultivation conditions. Overall, it is commonly accepted that apple trees fertilized with NPK and organic fertilizers present higher vigour and yield, being more capable of overcoming stressful conditions, such as altered climatic conditions (Milosevic et al., 2019). Given the high market demand for Royal Gala with the "Maçã de Alcobaça" PGI label, preserving fruit production while retaining nutritional qualities is crucial. Differential changes in

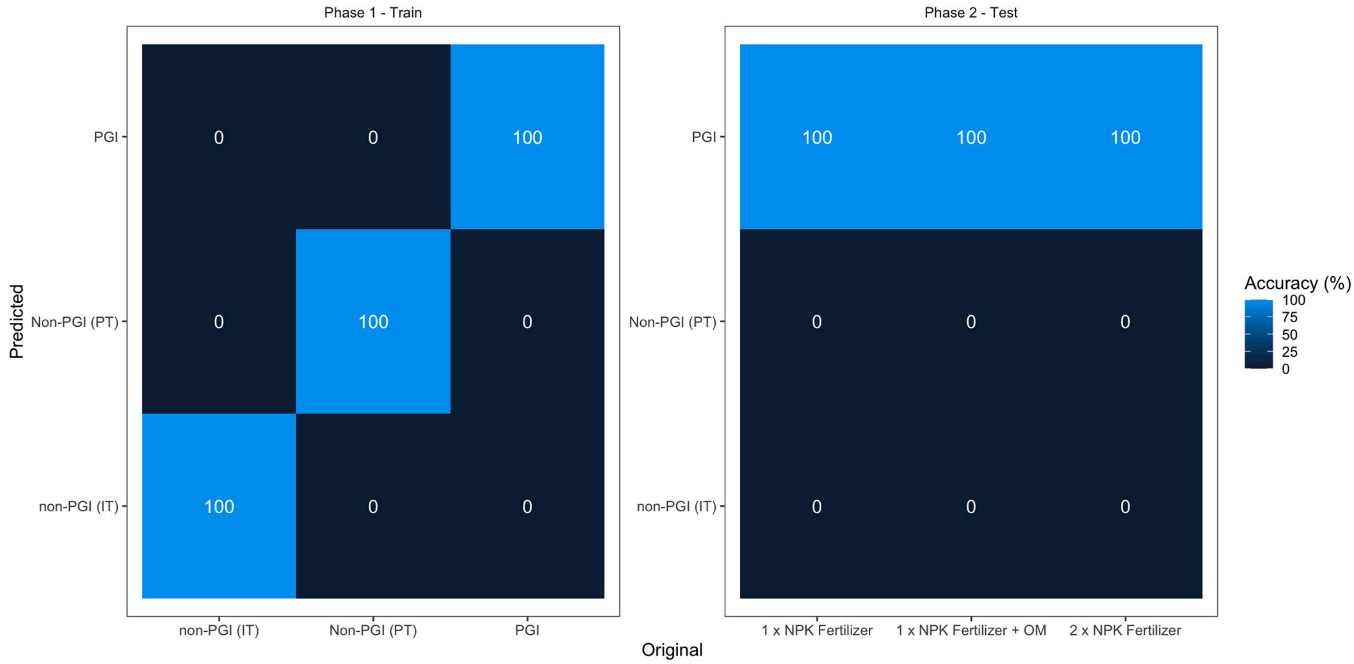

Fig. 2. Partial Least Squares Discriminant Analysis (VIP-PLS-DA) confusion matrix heatmaps of the training and test phases of the model having as basis the sample elemental profiles and according to their provenance [Protected Geographical Indication (PGI) and non-PGI from Portugal (PT) and Italy (IT)] and considering the applied fertilization practice.

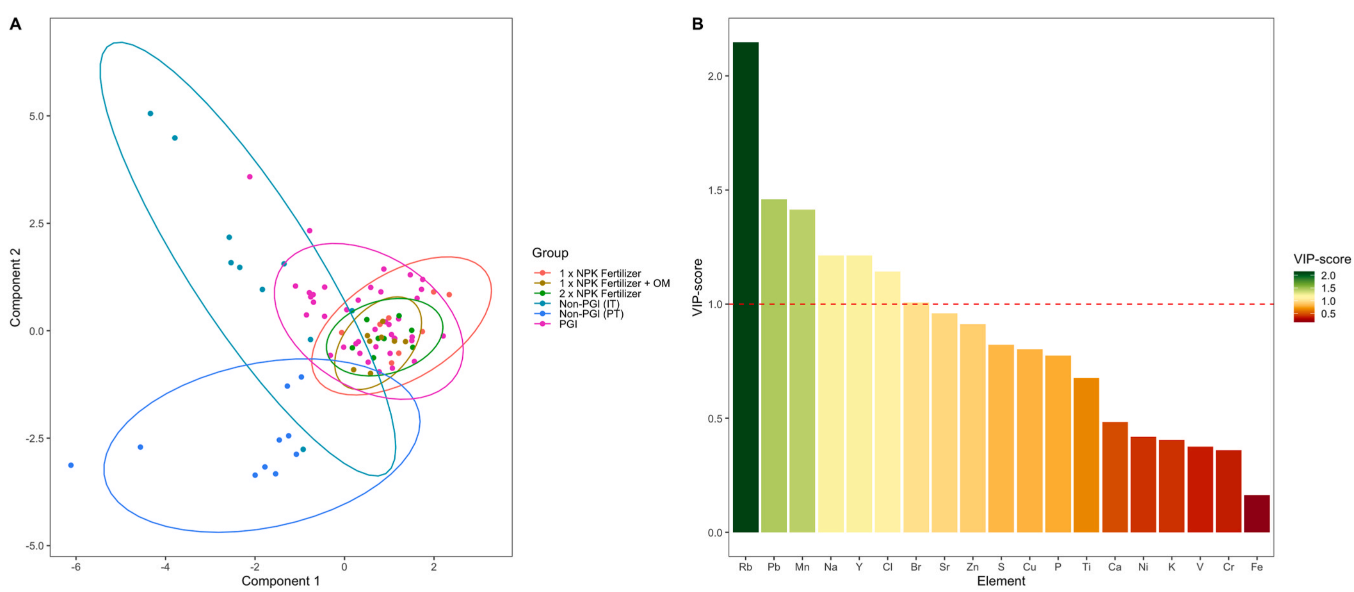

Fig. 3. Variable in Projection (VIP) Partial Least Squares Discriminant Analysis (VIP-PLS-DA) biplot (A) and element VIP-scores (B) of the apple pulp samples originated from PGI and non-PGI Portuguese (PT) and Italian (IT) orchards and trees subjected to the different tested fertilization treatments.

apple pulp elemental concentrations were apparent with varying fertilizer strategies. Calcium was one of the mineral nutrients that showed an increase in its concentration due to NPK fertilization. This is in agreement with previous studies that reported higher calcium uptake under NPK fertilization during fruit expansion and higher demand periods (Marti and Mills, 1991). Nevertheless, it appears that under higher NPK doses, calcium uptake is impaired when compared to NPK fertilization single application. This can be due to an antagonistic effect on the uptake of calcium by an overabundance of potassium in the soil, as reported on apple orchards (Dilmaghani et al., 2005; Neilsen and Neilsen, 2006). Regarding NPK fertilization coupled with organic amendment, it was previously reported that fertilization with swine manure increased the amount of calcium-bound organic carbon in the soil, which could potentially affect calcium uptake in plants (Wan et al., 2021). Previous studies have noted that NPK and organic-amended fertilization can boost the uptake of certain transitional metal elements. This occurs because these practices can slightly acidify the soil and weaken the binding forces of heavy metals to soil particles, thereby enhancing their solubility and mobility (Adewole et al., 2009; Singh et al., 2010). This was also observed in the present study for Mn, Ni, and Rb. Considering the elements also present in the fertilizer composition, namely K and P, there was an expected tendency for an increased concentration of these elements in the apple pulps upon NPK fertilization (Kuzin and Solovchenko, 2021). The exception was in the fruits harvested from trees where a reinforcement of the NPK fertilizer was applied, which presented a lower P and K concentration. Ródenas et al. (2019) found that high concentrations of K in the external solution inhibit inorganic P uptake and impair P nutrition in *Arabidopsis* plants, resulting in the induction of phosphate starvation response (PSR) and the upregulation of genes encoding root phosphate uptake systems. Since K is applied twice the concentration of P in the fertilizer application, the 2 x NPK treatment may have surpassed the K threshold concentration that leads to P uptake inhibition, leading to maintenance of the apple P concentration, similar to that observed under 1 x NPK fertilization. The iron content in apple fruits seems to have an inverse relationship with the NPK dose applied, with a significant reduction of the iron content in the analysed fruits harvested from trees subjected to 2 x NPK fertilization treatment. Previous works (Zheng et al., 2021) found that Fe uptake by *Malus hupehensis* plants decreased with an increase in K supply. Regarding Zn, the reinforced application of NPK fertilizers led to a decrease in the Zn fruit content. Previous works have reported decreases in Zn uptake and accumulation with increasing exogenous P application, although an increasing P concentration was observed in these treatments (Balafrej et al., 2020). This underscores the importance of optimizing P fertilization to enhance Zn content in plants. Nutritionally, the alterations in the mineral profile of apple pulp could be enhanced by applying 1 x NPK treatment. Consuming an average of 2 pieces of fruit per day (160 g each) could then fulfil a higher percentage of daily requirements for most essential elements crucial for human health. Also, noteworthy to mention, that none of the tested fertilization practices led to a reduction in the nutritional quality of the fruits analysed when compared to the 1 x NPK condition.

If on one side it is important to ensure that there is no loss of nutritional value under different fertilization practices, in terms of traceability, it is also of utmost importance to ensure that the elemental fingerprints of the fruits are maintained as characteristic of a “Maçã de Alcobaça” PGI product. Our previously developed models (Duarte et al., 2023b) have already shown that there is a clear differentiation between the elemental fingerprints of PGI and non-PGI apples from different sources (Portugal and Italy), arising as a potential tool to fight PGI food fraud (Barendse et al., 2019). The efficiency of these models is attributed to a set of elements that present high discriminatory power (VIP-score > 1) among the samples supplied to generate the models. Several of these elements are related to geological features inherent to the different production areas (PGI-area, North Portugal and Italy) (Inácio et al., 2008; Zhang et al., 2021). This is the case of Rb, Y and Pb, and although Pb was not detected in the samples analysed in the present fertilization assays its presence in samples from non-PGI areas, makes this element a discriminatory element. While some elements with strong discriminatory power lack known physiological roles (Marschner, 1995), their bioavailability in soils relies heavily on geological characteristics of the cultivation area. Typically unaffected by fertilization practices, they underscore their significance as discriminants in traceability models (Wyszkowski and Brodowska, 2020). Elements that exhibited substantial changes when comparing fruits from non-fertilized orchards to those from tested fertilization practices had lower discriminatory power. This underscores the influence of edaphic characteristics of the production area on apple elemental profiles, which can overshadow physiological traits of trees under various fertilization schemes. This becomes even more evident when evaluating the accuracy of the models produced with samples from PGI and non-PGI areas applied to the samples’ elemental profiles of the fruits gathered from fertilized orchards, as blind samples. All samples from the fertilization trials were consistently classified into the PGI category by the trained model during the test phase. This supports the hypothesis that the geological features of the cultivation area prevail over the influence of fertilization practices and intrinsic physiological traits (Duarte et al., 2023b; Wyszkowski and Brodowska, 2020). This is not only patent in the model accuracy results but also in the sample biplot distribution between the model’s two first components. Three evident clusters/sample groups could be observed: i) PGI samples overlaid with samples from the fertilization experiment; ii) samples from non-PGI areas from the North of Portugal; and iii) samples from non-PGI areas from the Italian production region of Trentino-Alto Adige/-Südtirol, in the country’s far north. Chloride and Br also share this behaviour with Pb and were also highlighted as elements with strong discriminatory power by the VIP-score analysis. Samples collected from fertilized orchards, independent of the treatment used, and from the INIAV core collection (as a certified PGI origin) share most of the elemental characteristics, being therefore grouped in the same cluster, reinforcing the low degree of impact of the fertilization practices on the PGI elemental signature.

5. Conclusions

The onset of climate change and soil degradation increases the need to adopt fertilisation practices that allow orchards to maintain productivity even under less favourable cultivation conditions. This is especially concerning when addressing PGI products with high demand and market value such as “Maçã de Alcobaça”, namely the Royal Gala cultivar, the one with higher market demand. Considering the three fertilization practices tested (1 x NPK, 2 x NPK and 1 x NPK supplemented with an organic amendment) and their impacts on the mineral content of the apple pulp it was possible to observe that, compared to non-fertilized trees, the fruit nutritional quality was not affected. Despite the changes in some mineral nutrients of the fruits, their overall elemental signature was still maintained under the different fertilization practices. Thus, the choice of the fertilization practice should be made according to the needs of the orchards to withstand less favourable culture conditions, as the nutritional value and elemental traceability signatures of the fruits are ensured among the tested fertilization practices. The results highlight the role of the edaphic characteristics of the cultivation area as a major influence on the elemental signature of PGI products and reinforce the use of elemental signatures as authenticity tools to fight food fraud and confirm the PGI provenance of the fruits.

CRediT authorship contribution statement

João Albuquerque Carreiras: Writing – review & editing, Methodology, Investigation. **Bernardo Duarte:** Writing – original draft, Resources, Project administration, Data curation, Conceptualization. **Marta Gonçalves:** Writing – review & editing. **Juliana Melo:** Writing – review & editing, Methodology, Investigation. **Miguel Leão de Sousa:** Writing – review & editing, Resources. **Anabela B. Silva:** Writing – review & editing, Resources.

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.

Data availability

Data will be made available on request.

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