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Handling traceability issues in food: a methodological approach for oenological products

a dissertation submitted for the degree of Doctor of Philosophy

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"I am among those who think that science has great beauty.
A scientist in his laboratory is not only a technician:
he is also a child placed before natural phenomena
which impress him like a fairy tale."

Marie Curie

"In science one tries to tell people,
in such a way as to be understood by everyone,
something that no one ever knew before."

Paul Dirac

Abstract

Nowadays, food quality and traceability play a primary role in the society mainly due to several episodes that threatened the authenticity and safety of foodstuffs. The oenological productions, owing to their peculiarities and global worldwide diffusion, show the greatest need for protection, valorization and innovation measures and could have the best growing opportunity. Since the quality of wine is in many cases related to the concept of *terroir* (history, geographical origin, typical raw materials, methods…), the possibility to assess the link between territory of origin and the food could represent a peculiar added value, useful for the enhancement of the product itself. Hence, the definition of objective criteria for the geographical traceability of wine, with particular attention to those awarded with quality marks such as PDO, PGI, etc., could represent a real challenging task.

The main research project, which this thesis belongs to, is focused on the development of geographical traceability models and operates with a twofold strategies: on one hand an extensive investigation is carried out on commercial wines and territorial matrices, and on the other hand a pilot study was started to clarify some critical points. In particular, the following aspects were taken into consideration: 1) identification of the suitable indicators, 2) optimization of the analytical methodologies for their determination, 3) study of their behavior with respect to soil variability, plant uptake and winemaking chain influence and 4) planning of a representative sampling for soils and food.

In this thesis, an approach based on the synergy between analytical methods and multivariate data analysis, was used to investigate some of the above mentioned aspects, in order to obtain information about the analyzed system.

In particular, to obtain more detailed information on the soil sampling procedure, in terms of inter and intra site variability, sampling depth and seasonal variability, a fast

screening approach by means of X-ray powder diffraction and multivariate spectra analysis on the soils was carried out.

After that, the $^{87}Sr/^{86}Sr$ isotope ratio was selected among the primary traceability indicators and evaluated in several matrices of the oenological chain. Sr isotope ratio varies according to the age of the rocks, the initial $^{87}Rb/^{87}Sr$ value and the elapsed time, thus being related to the geological characteristics of the territory. Moreover, Sr is involved in metabolic processes and should be absorbed by plant with no fractionation effects on the isotopic signature. Owing to these peculiarities, the $^{87}Sr/^{86}Sr$ should maintain the same value passing from soil to plant and then up to the bottled wine. However, several factors, related to anthropic or natural contaminations, plant uptake, making procedures, could affect the isotopic relative abundance and should be considered when investigating the transition from soil to wine. A Multi Collector ICP/MS was used to determine the $^{87}Sr/^{86}Sr$ in different matrices, soil – branch – grape juice – wine, to monitor the variability of the indicator verifying its effectiveness. Moreover, great effort was paid to rationalize the aspects related to the element uptake of the vine plant and, as a consequence, to determine the Sr isotope ratio on the bioavailable Sr fraction.

The results highlight a good match between the isotopic values monitored in the soil fractions mimicking the bio-available part and their respective grape juices for almost all the investigated geographical areas. The correlation with food matrices satisfyingly improves when the isotopic values of vine branches are considered.

Riassunto analitico

I concetti di qualità e tracciabilità alimentare ricoprono un ruolo primario nella società, principalmente a causa dei recenti casi che hanno minacciato l'autenticità e la sicurezza di alcuni prodotti. Le produzioni enologiche, per le loro caratteristiche e la vasta diffusione, necessitano di interventi per la protezione, valorizzazione e innovazione, da cui possono trarre opportunità di crescita. Poiché la qualità del vino è strettamente legata al concetto di *terroir* (storia, origine, metodi e materie prime tradizionali), il legame tra alimento e territorio di origine può rappresentare un valore aggiunto. Quindi, la definizione di criteri oggettivi per la tracciabilità geografica dei vini, con particolare attenzione per prodotti a denominazione (DOP, IGP, STG), può rappresentare una svolta innovativa. Il progetto di ricerca di cui fa parte questo lavoro è finalizzato allo sviluppo di modelli di tracciabilità geografica e opera con una duplice strategia: da una parte l'investigazione estesa di vini commerciali e matrici territoriali a dall'altra uno studio pilota per chiarire alcuni punti critici. In particolare, i seguenti aspetti sono stati presi in considerazione: 1) l'identificazione degli indicatori adatti, 2) l'ottimizzazione di metodologie analitiche per la loro determinazione, 3) lo studio del comportamento degli indicatori in relazione alle diverse fonti di variabilità (suolo, uptake della pianta, processo produttivo) e 4) la pianificazione di un campionamento rappresentativo per tutte le matrici.

In questa tesi, un approccio basato sulla sinergia tra metodi analitici e analisi multivariata è stato utilizzato per studiare alcuni degli aspetti sopra citati, al fine di ottenere informazioni sul sistema analizzato.

In particolare, è stato fatto uno screening dei terreni mediante diffrazione a raggi X e tecniche chemiometriche, per valutare in modo semplice e veloce la variabilità inter e intra sito, considerando l'influenza della profondità e del periodo di prelievo, ottenendo così indicazioni sulla procedura di campionamento (profondità e periodo, numero e posizione dei campioni all'interno dei campi).

In seguito, il rapporto isotopico $^{87}Sr/^{86}Sr$ è stato scelto tra gli indicatori analitici di tracciabilità e valutato in diverse matrici della filiera enologica. $^{87}Sr/^{86}Sr$ varia in base all'età delle rocce, all'iniziale rapporto Rb/Sr e al tempo trascorso, risultando quindi correlato alle caratteristiche geologiche del territorio. Inoltre, Sr è coinvolto nel metabolismo delle piante e risulta assorbito senza effetti di frazionamento sul rapporto isotopico. Per questo, il rapporto isotopico dello stronzio dovrebbe mantenersi costante dal terreno alla piante e infine nell'alimento. D'altro canto, diversi fattori (assorbimento della pianta, processo produttivo, contaminazioni antropiche o naturali) potrebbero influenzare l'indicatore e dovrebbero quindi essere considerati.

Un ICP/MS multicollettore ad alta risoluzione è stato utilizzato per determinare $^{87}Sr/^{86}Sr$ in diverse matrici (terreno – tralci – succhi) al fine di monitorare la variabilità dell'indicatore nel terreno e verificare la sua efficacia. Inoltre, grandi sforzi sono stati fatti nel razionalizzare gli aspetti legati all'assorbimento di elementi da parte della vite e, di conseguenza, determinare il rapporto isotopico nella frazione biodisponibile di stronzio. I risultati ottenuti evidenziano una buona corrispondenza tra i valori di rapporto isotopico misurati nelle frazioni di terreno, mimanti la parte biodisponibile, e i rispettivi succhi per quasi tutte le aree investigate. La correlazione con l'alimento migliora significativamente considerando i valori ottenuti per i tralci.

Table of abbreviations

Abbreviation	Description
BSE	Bovine Spongiform Encephalopathy
CEC	Cation Exchange Capacity
DOC	Denominazione di Origine Controllata (Italian wine denomination)
DOCG	Denominazione di Origine Controllata e Garantita (Italian wine denomination)
DoE	Design of Experiment
EU	European Union
F	PARAFAC factor
FAAS	Flame Atomic Absorption Spectroscopy
GC-MS	Gas Chromatography – Mass Spectrometry
ICP-OES	Inductively Coupled Plasma – Optical Emission Spectroscopy
ICP-qMS	Inductively Coupled Plasma – quadrupole Mass Spectrometry
IGT	Indicazione Geografica Tipica (Italian wine denomination)
IR	Infrared Spectroscopy
IRMS	Isotope Ratio Mass Spectrometry
LC-MS	Liquid Chromatography – Mass Spectrometry
MC-ICP-MS	Multi collector – Inductively Coulpled Plasma – Mass Spectrometry

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Abbreviation	Description
MCR	rectified concentrated must
MW	Microwave
NMR	Nuclear Magnetic Resonance
PARAFAC	Parallel factor analysis
PC	Principal Component
PCA	Principal Component Analysis
PDO	Protected Designation of Origin
PGI	Protected Geographical Indication
P.F.	loss on ignition
REEs	Rare Earth Elements
RSD	Relative Standard Deviation
SPE	Solid Phase Extraction
Sr-I.R.	strontium isotope ratio
TSG	Traditional Speciality Guaranteed
XRDP o XRD	X-ray diffraction of powder
XRF	X-ray fluorescence

List of publications

M. Cocchi, C. Durante, A. Marchetti, M. Li Vigni, C. Baschieri, L. Bertacchini, S. Sighinolfi, L. Tassi and S. Totaro, "Optimization of microwave assisted digestion procedure by means of chemometric tools", in *Microwaves: theoretical aspects and practical applications in chemistry*, A. Marchetti (Ed.), Transworld Research Network, Kerala, India, 2011, pp. 203 - 226. (ISBN 978-81-7895-508-7)

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G. Papotti, D. Bertelli, R. Graziosi, M. Silvestri, L. Bertacchini, C. Durante and M. Plessi, "Application of one- and two-dimensional NMR spectroscopy for the characterization of Protected Designation of Origin Lambrusco wines of Modena", *J. Agric. Food Chem.*, 2012, (http://pubs.acs.org/doi/abs/10.1021/jf302728b).

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CHAPTER 1

Introduction

1.1. The traceability issue: different points of view for the same goal

Quality and safety are two frequently discussed topics that are becoming more and more important for consumers, producers and regulatory institutions as well [1–3].

In the last decades, different episodes have contributed to decrease consumers' confidence towards food products and to draw the attention to what we eat, the provenance and the production methods. The dramatic increase in food health scares (BSE, dioxin, avian influenza, food contamination, food borne diseases) highlighted the weaknesses in both the production and the control chain and the need to obtain clear and detailed information about the used raw materials and the processing of food, in order to facilitate the identification of contaminating sources and thus the product recall. Besides the fundamental requirements of health and safety, other quality features can be related to product peculiarities, often linked to geographical origin or production area, to special ingredients or particular production methods, resulting from local expertise and traditions [3]. Consumers' interest in high quality food with a well defined or recognizable origin is thus associated not only with health and safety conditions, being these products perceived to be more controlled, but also with the feeling of *nostalgia* that reminds them of their roots [1, 4].

As a consequence, the production of specialty food in particular regions or using traditional methods could be considered as an added value. Producers of such foodstuffs could obtain economic advantages by responding to market demands of gaining more information regarding the history of the product, its geographical origin, the raw materials employed in its production, the chain process, etc. [2].

In this context, the European Union (EU) played a key role by recognizing the link between the product and the territory of origin as quality attribute. Moreover, the promotion and protection of typical food and beverages and the improvement of quality standards, reinforcing control system throughout the food chain, are certainly topics of great importance for the EU. This quality policy started in 1992 by adopting two regulations [5, 6], further amended in 2006 and 2008 [7–10], which introduce product

designations, namely Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Speciality Guaranteed (TSG).

The first two marks identify products whose quality, characteristics or reputation are strictly linked to a specific territory, whereas the last one is mainly related to a peculiar production method. In particular:

- ✓ **Protected Designation of Origin (PDO):** indicates food with proven features resulting from the territory of origin and the skills of producers in the region with which they are associated. All the steps of the production process have to be carried out in the specified region.
- ✓ **Protected Geographical Indication (PGI):** refers to products with specific characteristics or reputation relating them to a given area. At least one production stage must occur in that region.
- ✓ **Traditional Speciality Guaranteed (TSG):** characterizes products with distinctive features associated to traditional ingredients or production methods, but it is not related to a specific geographical area.

Both consumers and producers can capitalize on these designations, which allow identifying high quality products and guaranteeing their genuineness and authenticity. Notwithstanding the regulations, aliments with a high reputation and added value are usually prone to frauds or counterfeiting. Therefore, the protection of genuine and authentic specialty foodstuffs from the attacks to their reputation and the exploitation of their names is a major concern for the EU and should be constantly seek by a careful quality management system.

The EC 178/2002 [11] sets out the basis for a new control method by defining the terms of traceability and production chain traceability. Traceability is defined as 'the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be, incorporated into a food or feed, through all the stages of production, processing and distribution. Therefore, it should be possible to obtain information relative to every step from the farm to the final product (tracing) and also to go back from the consumer to the food origin (tracking).

This form of traceability, that we might call "conventional traceability" is almost based on the papery documents and is aimed at identifying each product or product batch, keeping track of every occurring process action [12, 13]. Main aim of this system is to provide targeted information to increase safety and fasten product recall in case of food emergencies.

Beside this, the geographical traceability is aimed to assess the geographical origin of products. This is particularly useful for special food awarded with the Protected Designation of Origin, being these products produced in a specified area with local raw materials and typical production methods. Hence, traceability could represent a powerful tool to help to establish the authenticity of such food and to check that claims made by producers about food are true.

Unfortunately, many of the actual traceability systems are generally not referred to objective criteria but mostly based on certifications supported by papery declarations. Thus, in a context of globalized market and international food trade, with high risk of imitation, counterfeiting and adulteration, the possibility to trace the provenance of food on the basis of objective analytical criteria could be certainly a valuable support for the traditional papery declarations.

This method would allow to clearly documenting the link between the food and the region of origin, enhancing the existing quality and traceability systems and reinforcing the confidence in food and food industry.

1.2. The scientific researches towards geographical traceability

The analytical indicators, which can allow investigating the provenance of food, can be divided into two categories [14]:

- ✓ **primary or direct indicators**: variables able to directly link some chemical characteristics of the territory with the same ones measured in the final products. Among these, the elemental composition, the stable isotope ratios of light

elements (H, C, N, O and S) and the ratio of the relative isotopic abundances of radiogenic heavy elements (Sr, Pb, Nd) are the most used;

- ✓ **secondary or indirect indicators**: variables related to compositional properties of the food and to the transformation process, which allow, through an extensive characterization of the matrix, to identify products with the same origin, while discriminating from the different ones. Spectroscopic and spectrometric techniques, such as IR, NMR, GC-MS, LC-MS, etc., are the most used to obtain signals related to the composition of the food matrix.

In recent years, there were several scientific attempts to develop food traceability and authenticity models. In particular, the growing interest in this topic is demonstrated by the large number of reviews, regarding different kinds of food and beverages, such as honey [15, 16], wine [17, 18], meat [19, 20] and dairy products [21], and different analytical tools [22–27].

In the last decade, the main effort to assess the potentiality of most innovative analytical techniques for geographical traceability of food commodities was carried out in the VI European framework by the TRACE project [28]. Different European commodities, such as mineral water [29], olive oil [30], meat [31], honey [32] and cereals [33], were investigated with promising results, by means of both primary and secondary indicators.

However, the adopted large scale approach was not sufficient to face specific problems on limited regions and TRACE conclusions highlighted the need to focus on more localized geographical areas, in order to really assess the effectiveness of the geographical markers. Indeed, although they seem promising, these indicators require more detailed studies to be verified as possibly suitable in a control protocol.

Further, when dealing geographical traceability, it is essential to consider the reality in which the food lies, in order to develop robust models perfectly fitting the problematic and being in line with the final aims. Products awarded with Protected Designation of Origin and typical of extensive areas require *large scale* models, considering a representative mapping of the territory as well as a systematic knowledge of the

foodstuffs. On the contrary, specialty products, characteristic of restricted areas or linked to the producer's brand, need more detailed traceability models.

Finally, in traceability studies, three different analytical approaches can be mainly adopted in order to link the food commodities to the territory of provenance. The first one is based on the measurement of traceability indicators in a representative number of food samples. The link between the food and its geographical origin is found by using multivariate models and considering a 'training set' of samples with guaranteed geographical origin and authenticity. In the second approach, the primary indicators are directly measured both on the investigated food as well as in some reference samples of their soils of origin, without systematic selection of soils. The third approach consists in the determination of indicators in food and in a representative set of soil samples, selected by taking into account climate, geographical and geological features. The choice of one approach over another mainly depends on the aim of the research and on the posed question too. In any case, it is of utmost importance to build models that take into account the cause/effect relationship among the monitored variables.

1.3. Aims and outlines of the thesis

The overall description of the current socio-economical situation and the European policy regarding the food traceability issue, described in the previous sections, highlighted the need to develop analytical tools to trace the origin of food.

In fact, the definition of objective criteria, able to certify the authenticity of a product and to trace its production process, including the possibility to obtain a geographical and varietal traceability, is certainly a challenging task, which could have important effects both from an economic and social standpoint. This is particularly relevant for food awarded with quality designations related to geographical origin, since this approach could support the existent system in the protection of high value products.

Italy is known as one of the main European country in terms of value of specialty production. In particular, Italy boasts the European leadership as regards PDO/PGI food products (wine excluded) with 244 denominations. As far as wines are concerned, Italian regulations classify high quality productions on the basis of three traditional denominations, namely Denominazione di Origine Controllata e Garantita (DOCG), Denominazione di Origine Controllata (DOC) and Indicazione Geografica Tipica (IGT). Recently, these denominations were updated to PDO and PGI according to the European policy, even if the traditional marks can be maintained [34, 35]. In the European Community, 1321 PDO and 585 PGI wines are acknowledged; Italy follows France with 403 PDO and118 PGI products [36].

Among the several productions in the food sector, the enological one results to be of great relevance for the Italian economy, because of its peculiarities and worldwide diffusion. For these reasons, the wine sector could benefit from protection and innovation measures and obtain the best opportunity for a new growing.

The research activity, here presented, is part of a long term project (AGER 2009 [37]), which deals with the development of analytical methodologies for the authenticity and geographical traceability of Italian oenological products by means of objective parameters (traceability indicators). Examples from other parallel studies are also presented.

The proposed approach is based on the synergistic use of both advanced analytical – instrumental facilities, to obtain data characterized by high accuracy and precision, and multivariate data analysis tools, which allow an optimized, efficient and low time consuming data analysis.

The way to obtain reliable traceability models is actually quite long and difficult, since many different matters should be taken into account, i.e. the optimization of the analytical procedures for the determination of the indicators, the planning of a representative sampling both for soil and food matrices, the investigation of the indicators' behavior in the different matrices of the production chain, etc.

Considering the great number of variables affecting most of these issues, it is noteworthy that the use of chemometrics techniques is of fundamental support to obtain a clear and comprehensive understanding.

Starting from the considerations arising from the TRACE project [28], this work will focus on localized regions, taking into account some peculiarities related to the traceability topic and in particular to the $^{87}Sr/^{86}Sr$ isotopic ratio, which proved to give excellent results for different matrices [20, 22, 26, 27, 29, 33].

In Chapter 2 an overview of the potentialities of $^{87}Sr/^{86}Sr$ isotopic ratio as geographical tracer is presented. In particular, the strontium isotope pattern is described in order to better understand its uses and potentialities. Then, the variability of strontium isotopic ratio in major rock types, the several sources and relationships which characterize the transfer from rock to soil and then plant are taken into account. Finally, different issues related to the use of this indicator for traceability studies are highlighted, also considering the state of the art.

As stated before, when starting to develop geographical traceability models, it is of utmost importance to optimize all the analytical procedures necessary for the indicators determination. This step was earlier handled in two Doctoral theses [38, 39]. After, the attention has to be focused on the planning of the sampling procedures, since their representativeness inside the considered area greatly influences the robustness and reliability of the obtained models. The selection of the soil sampling sites was performed by means of Design of Experiment tools, taking into account several variables related to the location and dimension of the sites, their geological features as well as productivity aspects [38]. A basic knowledge of the investigated territory could be of great support in order to decide the appropriate approach, namely location and depth of the soil samples and the suitable sampling period. In Chapter 3, the use of X-ray diffraction of powder (XRDP) coupled with chemometrics techniques is investigated as fast and simple screening tool for assessing soil samples variability, in terms of geological features. In this way, it is also possible to obtain a rational

standpoint on the basis of which reduce the number samples to be characterized by other more costly analytical techniques.

Besides the sampling procedure issues, the transfer process of the $^{87}Sr/^{86}Sr$ fingerprint from soil to vine and then up to the final product was investigated as well. Firstly, in Chapter 4, the $^{87}Sr/^{86}Sr$ variability in soil is monitored, in order to verify the inferences coming from the XRDP study. Then, the behavior of the indicator in the soil-grapevine system is examined, by evaluating the issues related to the complex structure and composition of soils and to the determination of the biologically available fraction. The use of the plant as direct sampling tool is suggested in order to overcome these problems and obtain a better correlation with the grape juices.

Finally, in Chapter 5, the influence of different winemaking processes on the $^{87}Sr/^{86}Sr$ values is discussed with the aim of obtaining a geographical traceability for the final product.

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CHAPTER 2

Strontium isotope ratio as geographical traceability marker

2.1. Strontium isotope system

Strontium (Sr), like the other elements of Group II, such as beryllium (Be), magnesium (Mg), calcium (Ca) and barium (Ba), is a divalent alkaline earth element. Owing to its properties (in particular the ionic radius of Sr^{2+} is 1.18 Å), quite similar to those of calcium (Ca^{2+} ionic radius is 1.00 Å), Sr easily substitutes for Ca in a wide range of minerals including plagioclase feldspar, apatite, sulfates such as gypsum and anhydride, and carbonates (calcite, dolomite and especially aragonite). Strontium has four natural stable isotopes: ^{84}Sr (~ 0.56%), ^{86}Sr (~ 9.87%), ^{88}Sr (~ 82.53%), which occur in constant relative proportions, and ^{87}Sr (~ 7.04%), which increases over geological time. In fact, among strontium isotopes, only ^{87}Sr is radiogenic, since it is formed over time by the β^-decay of ^{87}Rb, with a half-life of about 4.8×10^{10} years. Rubidium is an alkali metal, which is highly present in K-bearing minerals (muscovite, biotite, alkali feldspars, clays, evaporites), since its ionic radius is only slightly bigger than that of K^+. [1, 2].

The Rb-Sr decay system is described by the Equation 2.1, meaning that a ^{87}Rb atom produces a ^{87}Sr atom, two nuclear particles, a beta particle (β^-) and an anti-neutrino ($\bar{\upsilon}$), and decay energy (Q) as kinetic energy of the particles.

$$^{87}Rb \Rightarrow {}^{87}Sr + \beta^- + \bar{\upsilon} + Q \qquad (2.1)$$

Since, as a variant of the general radioactive decay equation, the remaining ^{87}Rb is given by $^{87}Rb = {}^{87}Rb_0\, e^{-\lambda t}$, where $^{87}Rb_o$ is the initial amount, λ the decay constant for ^{87}Rb (1.42×10^{11}) and *t* is time, the present ^{87}Sr can be represented by Equation 2.2.

$$^{87}Sr = {}^{87}Sr_0 + {}^{87}Rb_0 - {}^{87}Rb_0\, e^{-\lambda t} \qquad (2.2)$$

where $^{87}Sr_o$ is the original amount of ^{87}Sr at $t = 0$.

Usually, rather than considering only the ^{87}Sr abundance, the isotopic abundance ratio, $^{87}Sr/^{86}Sr$ (Equation 2.3), is preferably used as geochemical tracer or for geochronological purposes.

$$\frac{^{87}Sr}{^{86}Sr} = \left(\frac{^{87}Sr}{^{86}Sr}\right)_0 + \frac{^{87}Rb}{^{86}Sr}\left(e^{\lambda t} - 1\right) \cong \left(\frac{^{87}Sr}{^{86}Sr}\right)_0 + \frac{^{87}Rb}{^{86}Sr}\,\lambda t \qquad (2.3)$$

From Equation 2.3 emerges that $^{87}Sr/^{86}Sr$ isotope ratio in a rock depends on: i) the $^{87}Sr/^{86}Sr$ at the time the rock crystallizes (t = 0), ii) the $^{87}Rb/^{86}Sr$ ratio of the system, which, in most cases, is directly related to the Rb/Sr concentration ratio, iii) the decay constant λ of ^{87}Rb and iv) the time t elapsed since formation [3].

As regards the continental crust – mantle system, Rb is a highly soluble, highly incompatible element. Sr is also relatively soluble and not quite as incompatible, because of the smaller ionic radius; moreover, it is relatively compatible in silica-rich igneous systems, partitioning preferentially into plagioclase [2, 3].

Owing to these properties, during the formation of the continental crust from the melted mantle, a relatively high amount of Rb was incorporated in the liquid phase with respect to what was originally in the solid mantle. As a result, the continental crust has, on average, more radioactive ^{87}Rb than the mantle and, over time, it will potentially accumulate more radiogenic ^{87}Sr.

The *isotope evolution diagram* ($^{87}Sr/^{86}Sr$ vs. time) in Figure 2.1 shows the Sr isotope variation in the earth and its major silicate reservoirs (continental crust and mantle).

The Earth's crust, produced by partial melting and crystal fractionation processes of the mantle, presents a wide range of relatively steep trajectories of $^{87}Sr/^{86}Sr$ values over time. This heterogeneity reflects the great variety of magmatic, sedimentary and metamorphic evolutionary processes and thus the variability in the Rb/Sr values [4].

Rb and Sr are incorporated into rocks during their formation on the basis of their characteristics and, over time, the amount of ^{87}Sr increases as radioactive ^{87}Rb decays.

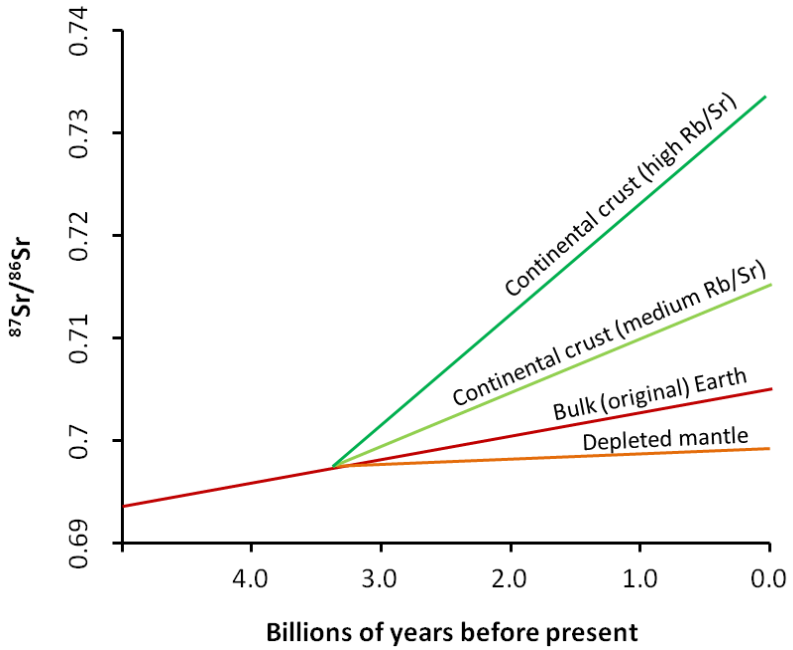

Figure 2.1. Sr isotopic evolution of the bulk Earth, high and low Rb/Sr crust and depleted mantle.

As a consequence, older rocks (> 100 mya) with high Rb/Sr ratios have higher $^{87}Sr/^{86}Sr$ ratios than the younger ones with low Rb/Sr ratio. In particular, $^{87}Sr/^{86}Sr$ in rocks varies between 0.702 and 0.750 and older granites generally have ratios above 0.710, while younger basalts' ratios are around 0.703 [2, 5].

Thus, strontium isotope ratio is an indicator of both age and geochemical origin of rocks.

2.2. Strontium isotope ratio in environment, soils and plants

The property of strontium isotopic abundance ratio, conventionally expressed as $^{87}Sr/^{86}Sr$ ratio [5], to be related to the age and the geochemical origin of rocks gave rise to a number of researches in different fields, such as investigation of the mantle

processes [4], study of the prehistoric migrations [2], geochronological dating [6] and tracing food origin [7–10].

In particular, different studies have provided that $^{87}Sr/^{86}Sr$ represents an optimal geographical fingerprint for food and animals, since strontium is actively involved in metabolism of bio-organisms (as substitute for calcium) and biological processes in plants should not fractionate strontium isotopes. On the other hand, mass-dependent fractionation of Sr isotopes, whether natural or instrument induced, is corrected for during mass spectrometric measurement by normalization of the non-radiogenic isotopes, $^{86}Sr/^{88}Sr$, to known values.

The time scale of these geological and biological processes is too short to allow changes in the $^{87}Sr/^{86}Sr$ value through the ^{87}Rb decay; besides, negligible isotopic fractionations occur when the Sr isotopes pass from rock to soil and then into biological solutions, because of the small relative mass differences of the isotopes [11].

Nevertheless, even if a rough estimation of the $^{87}Sr/^{86}Sr$ variation can be done by considering a geological map of the bedrock types and ages, the direct use of this value, in many cases, is not enough in traceability study, since it could not be representative of the $^{87}Sr/^{86}Sr$ entering the environment [12].

In fact, several factors may influence the biologically available fraction of the metal; hence $^{87}Sr/^{86}Sr$ taken up by plants not always is the same of the soil in which they grow, potentially resulting in values measured in soils or plants not being the same as those of the underlying solid geology [2, 5].

First of all, it should be considered that a single rock can be constituted by different minerals with $^{87}Sr/^{86}Sr$ ratios varying within a wide range, leading to a final rock value which depends on the proportion of its constituents. For example, a granitic rock can be made by two different feldspars: one mostly containing calcium, and hence strontium, with very low rubidium and thus $^{87}Sr/^{86}Sr$ close to 0.70; the other one, a potassic feldspar, having high Rb and low Sr content, resulting in $^{87}Sr/^{86}Sr$ values sometimes higher than 1. Moreover, these minerals can weather at various rates, having different impacts on the final isotopic signature. As a result, the weathering process can bring to values representative of the whole original substrate or only of specific peculiarities.

At the same time, the strontium isotope ratio of a geographical area should be considered as a mixture of various sources. Besides the weathering of rocks and soils, strontium can derive from sediments carried by stream waters as well as from atmospheric deposit [5, 13, 14].

In particular, most of the sediments are carried by rivers and are mainly due to the erosion of rocks in elevated position, since they weather faster than the plains. Usually, elevated areas are characterized by rocks from the younger crust, leading to sediments with a lower $^{87}Sr/^{86}Sr$ ratio [3]. At high elevation, indeed, the correlation between $^{87}Sr/^{86}Sr$ in bedrocks and in stream water is quite good; on the contrary, the $^{87}Sr/^{86}Sr$ of lower areas is influenced by the local bedrock but also by upstream sediments and precipitations [15].

Moreover, the strontium isotope ratio, $^{87}Sr/^{86}Sr$, in soils varies as function of the sampling depth, because of the above-mentioned mineral variability but also due to a variable balance of the different strontium sources along the vertical profile [16, 17]. As a matter of fact, as depth increases, the contribute of the bedrock weathering gains more relevance with respect to the atmospheric deposits.

At this point, it is clear that soils can present a wide range of $^{87}Sr/^{86}Sr$ ratios, according to the different weathering and proportion of minerals and to the mixing of various inputs (bedrock weathering and atmosphere deposit) and outputs (soil weathering).

Furthermore, as previously mentioned, it is important to make a distinction between the $^{87}Sr/^{86}Sr$ ratio of the soil and that one of the biologically available fraction, since the plants are not always able to take up the whole element fraction present in the soil where they are planted. In particular, in the soil, elements exist as:

- ✓ simple or complex inorganic ions, in solution or adsorbed on solid surfaces;
- ✓ ions forming soluble or insoluble complexes with organic ligands;
- ✓ impurities within a crystal lattice;
- ✓ precipitates, etc.

Their behavior, and hence the facility in the plant uptake, depends on which form they are present in the soil. On the basis of element mobility, it is possible to classify soil fractions as:

- ✓ soluble fraction: ions, molecules and chelates in solution, which represent the immediately available element fraction;
- ✓ exchangeable fraction: which has a lower availability with respect to the previous one, even if in a short time;
- ✓ available reserves: elements of simple mineral or organic structures, which are available after some time,
- ✓ not available reserves: elements constituting crystalline structures of minerals resistant to alterations, which are slowly available in a long time.

However, the evaluation of the bio-available fraction, namely the element part able to directly interact with plant receptors and then to be absorbed, is troublesome. It involves various difficulties related to the plant uptake, which depends not only on the elements concentration in the soil, but also on its geomorphologic and chemical characteristics as well as on the plant features.

Several studies have been carried out to evaluate the bio-available element fraction in soil, taking into account different extraction media [18, 21].

In principle, no methods are able to perfectly reproduce the real plant uptake in every circumstance, but there are some standard procedures, such as the DIN ISO 19730 [22], which are able to estimate the bio-available fraction.

On the other hand, some researchers have highlighted the possibility to directly use plants (or animals) as a good proxy of the local environment, since they allow assessing the bio-available isotopic signature, taking into account the multiple sources of strontium and averaging the soil variability [12].

2.3. Application of strontium isotope ratio for food traceability purposes

The application of strontium isotope ratio, $^{87}Sr/^{86}Sr$, for the geographical traceability of food products hinges in first approximation on the assumption that the characteristic isotopic signature of the bedrock is transferred to soil, plant and then up in the food chain, without further modifications (fractionation), allowing recognizing the original geographical provenance of the studied food.

Since additional sources of strontium (rainwater, aerosol, fertilizers, etc.), not always directly related to the local geology, occur and the plant uptake is somehow selective, the key point is to find a link between the strontium isotope ratio of a food and the biologically available signature of the territory of origin.

Several studies on different foodstuffs, wines, meats, cereals, etc., show the potentiality of $^{87}Sr/^{86}Sr$ as geographical tracer [23–29]. Some of these [24, 27, 28] relate the Sr isotope ratio of the aliment with that of the local environment (underlying rock, soil, water), measuring the investigated parameter in the food as well as in the soil fraction better mimicking the biologically available strontium. However, some discrepancies occurred, related to different factors, such as contributes of atmospheric sources or fertilizers or peculiarities in the plant uptake, as also discussed in Chapter 2.2, highlighting the need to have a deep knowledge of the investigated system (i.e. geology and environment, cultivation type…).

As regards the geographical traceability of wines, the early studies of Horn et al. [9] [10] shown a significant correlation between the value of $^{87}Sr/^{86}Sr$ in wines and their respective soils. Further, other researches confirmed the ability of $^{87}Sr/^{86}Sr$ to discriminate wines according to different geographical origins [29–31] and assessed that the winemaking procedure does not affect the indicator, if no "extraneous" strontium is introduced [32].

However, a systematic approach, considering a representative set of both soil and food samples, has not been adopted so far. The above mentioned researches generally involved restricted areas or a reduced number of samples for extended geographical

regions. Moreover, in some cases, the $^{87}Sr/^{86}Sr$ ratio was measured only of food matrices, disregarding to evaluate the link with the territory. Notwithstanding the potentiality of $^{87}Sr/^{86}Sr$ isotope ratio as geographical marker, these methods were not sufficient to face specific problems, which occur when dealing with more detailed geographical traceability models. In this context, an extended study about the features of strontium isotope ratio for wine provenancing, taking into account the different factors affecting the indicator, could help obtain more robust traceability models.

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CHAPTER 3

Fast screening of soils through the synergistic use of analytical techniques and chemometrics tools

3.1. Introduction

A critical step of any scientific investigation is the choice of the correct strategy to sampling procedure, even much more when dealing with the development of geographical traceability models for PDO (Protected Designation of Origin) food products. As a matter of fact, in order to obtain robust models based on traceability indicators able to give account of the direct cause-effect relationship between soil of origin and food, the accurate and representative sampling of all the involved matrices is of utmost importance.

Several approaches have been proposed in literature to plan soil sampling [1–3], e.g. make use of regular and circular grids, systematic and non-systematic patterns, unaligned random sampling, etc. Nonetheless, the aim of these methods is generally to uniformly sample restricted areas, considering only few parameters, mostly the geographical coordinates. Other approaches, mainly based on multivariate techniques, are able to take into account different characteristics of the investigated area, related to geological features as well as productivity information [4–6]. However, before starting any sampling, some parameters should be set, i.e. the number of samples, the period of sampling, the procedures and so on, always according to the aim of the study. The considered traceability indicator, the $^{87}Sr/^{86}Sr$ isotope ratio, is highly affected by the geochemistry and thus by the nature and composition of soil, and can vary greatly both inter- and intra- sites as well as along the vertical profile [7, 8], as described in Chapter 2. So, a basic knowledge of the different features of the territory and the investigation of the soil homogeneity could be of great support in order to choose location and depth of samples to be collected.

The determination of the strontium isotope ratio requires complex procedures [9–11]. The main steps include an appropriate extraction or digestion of the samples [7] and other treatments, such as resin separation, in order to obtain proper solution for the following instrumental determinations and to remove isobaric interferences [9, 10]. Due to the considerable time, effort and costs required for this sample preparation and for the MC-ICP-MS measurements, the mapping of the whole investigated area through the

determination of such indicator is not an affordable task. The possibility to find out a fast screening methodology for the evaluation of the sampling depth influence, field homogeneity, seasonal and time variability would be of great support in order to plan the soil sampling procedures, selecting a feasible number of soil samples to be collected, where the traceability indicator has to be determined.

The X-ray diffraction of powder (XRDP) is suggested as a preliminary technique capable to perform a blind analysis on soil samples. As a matter of fact, it permits to obtain a fingerprint, related to the different composition and morphological structure of the soils, by means of simple and relatively fast determinations.

Chemometrics plays a key role, since it allows extracting relevant information from the whole signals, without the need of identifying and quantifying *a priori* the amorphous and crystalline components that characterize them. Hence, X-ray diffractograms were unusually treated as a set of numerical variables and analyzed by means of chemometrics techniques. This exploratory analysis allows highlighting the similarities and differences among soil samples, merely considering the signals.

Since no previous attempts are found in literature, the aim of this work is to assess the potentiality of the proposed screening technique in assessing soil samples variability, in terms of geochemical features, in the context of food geographical traceability.

A pilot study, which considers four *Lambrusco* production sites, representative of the Modena district, was carried out focalizing the attention on a reduced number of samples, in order to improve the knowledge about the investigated system.

The fingerprint profiles obtained by XRDP analysis of soil samples were deciphered by multivariate and multi-way data analysis, i.e. PCA and PARAFAC [12]. This fast and non-destructive analytical approach helps highlight the differences among the samples and the sources of variability (inter- and intra site, along the depth profile and among the sampling periods), also giving indications about the soil features responsible for this. Moreover, the soil samples were discriminated on the basis of their XRD spectra, producing a pattern which well matches with those obtained by characterization with other more costly and time consuming analytical techniques, such as FAAS, ICP-OES, ICP/MS, XRF, determination of isotope ratios. Thus, the proposed methodology

provides a rational starting point for the reduction of the number of soil samples to be collected and further analyzed, still preserving an exhaustive description and representativeness of the investigated region.

3.2. Sampling and samples

In the present study, the case of *Lambrusco* wines was considered, when investigating the challenges related to soil sampling procedures for traceability purposes.

Lambrusco wines are PDO products, characterized by stringent production regulations [13–15] which allow the grape cultivation in the whole Modena district. This territory is located in the centre-north of Italy and comprises an in-plain area, centre – north part of the district, and a moderate/medium hill in the southern region. Po, Secchia and Panaro rivers represent the northern left and right boundaries of the area and the alluvial basins of these watercourses strongly influence the pedo and lithological features of the territory. The investigated area results to be quite extended, including 4062 producers and 90 km^2 of cultivated fields, and presents peculiarities which characterize the different places. For these reasons, a pilot study has been activated for the careful evaluation, on a reduced scale, of the suitable sampling conditions and procedures. Four farms, representative of the investigated area and including the whole production chain from the raw materials to the final products, were selected, taking also into account the greatest possible variability in the geological/pedological characteristics of the soils they insist on.

Figure 3.1 (*a*) represents the Modena territory where the cultivation of the grapes for the *Lambrusco PDO* wines is allowed, whilst (*b*) reports a pedological map of the Modena district. The four letters indicate the position of the chosen producers/fields; three of these (A, B and D) are located in an in-plain region, whilst the other one, producer C, is located in hill area. The colors of the areas highlight the different geological characteristics of the soil [16].

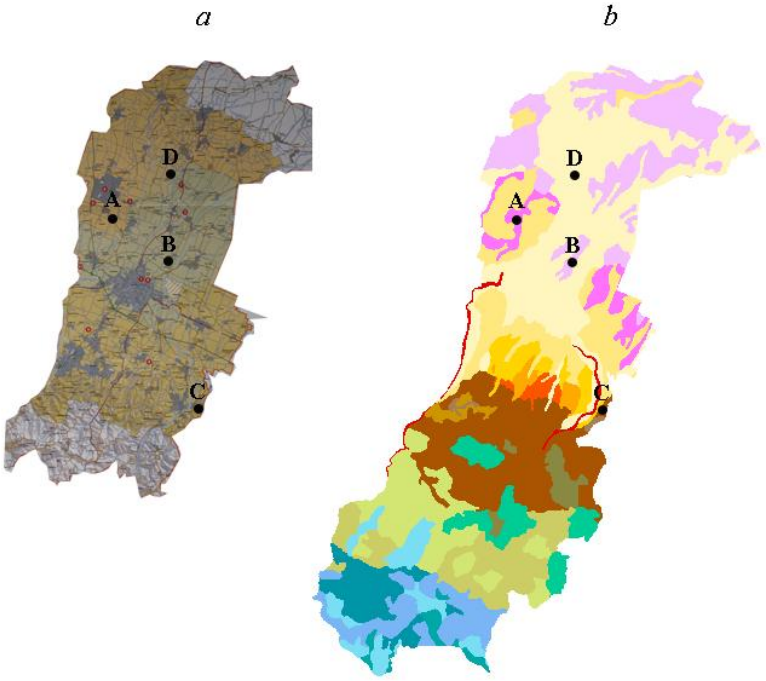

Figure 3.1. Position of the chosen producers/fields in the proper area of production of the *Lambrusco PDO* wines (*a*) and in the pedological map of the Modena district (*b*).

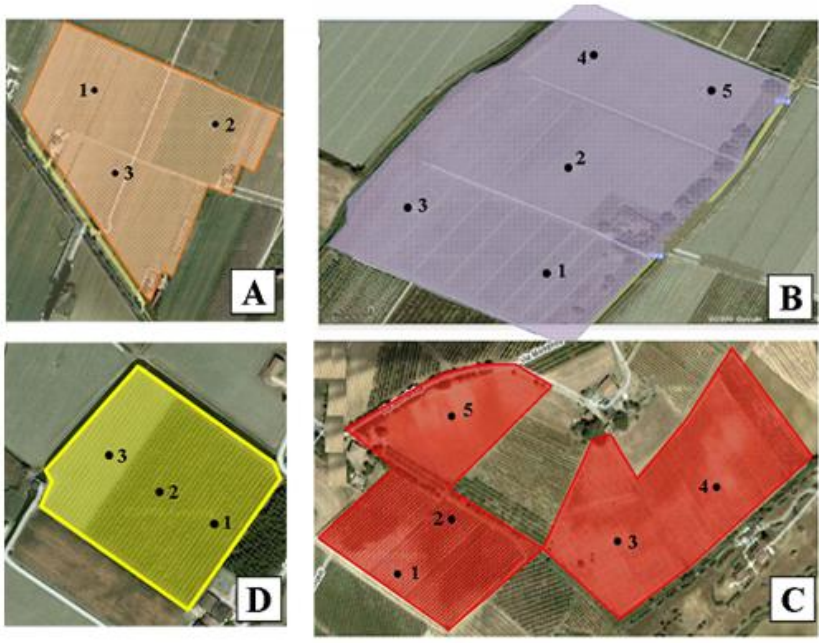

Figure 3.2. Sampling points for each producer/field of the pilot study. In particular, three sampling points are chosen for A and D, whilst five points for B and C.

Table 3.1. GPS position (degree, minutes, seconds) of the sampling points.

	Point 1	Point 2	Point 3	Point 4	Point 5
A	N 44° 44' 48.34" E 10° 53' 2.98"	N 44° 44' 46.86" E 10° 53' 9.71"	N 44° 44' 41.14" E 10° 53' 8.70"		
B	N 44° 42' 25.49" E 11° 00' 58.21"	N 44° 42' 30.20" E 11° 00' 59.33"	N 44° 42' 27.86" E 11° 00' 51.30"	N 44° 42' 35.14" E 11° 01' 1.02"	N 44° 42' 33.73" E 11° 01' 4.84"
C	N 44° 29' 6.04" E 11° 02' 57.80"	N 44° 29' 6.76" E 11° 02' 58.70"	N 44° 29' 6.76" E 11° 03' 8.96"	N 44° 29' 9.24" E 11° 03' 13.93"	N 44° 29' 10.90" E 11° 03' 1.84"
D	N 44° 46' 9.01" E 11° 00' 42.66"	N 44° 46' 11.71" E 11° 00' 40.75"	N 44° 46' 12.29" E 11° 00' 38.59"		

Three to five soil samples were collected within each field, on the basis of its extension, as shown in Figure 3.2. The GPS positions of the 16 sampling points are reported in Table 3.1. The soil sampling was performed by using a manual percussion single gauge auger (3 cm internal diameter) set for hardly disturbed samples. Five soil aliquots were sampled, starting from a depth of 10 cm up to 60 cm (Figure 3.3). The superficial part (0–10 cm) was eliminated to avoid the possible presence of grass or debris. The deepest value was set to 60 cm because most of the grapevine root system is found above that limit [17].

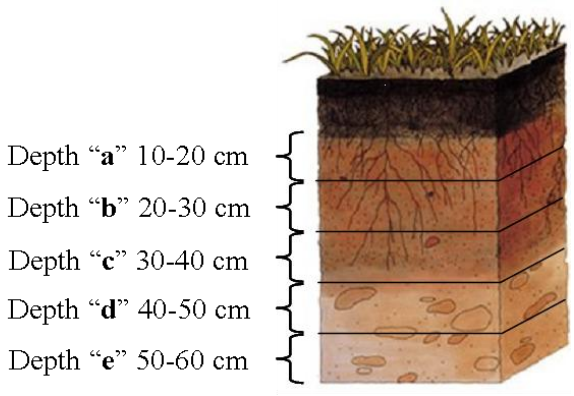

Figure 3.3. Representation of the five soil aliquots, sampled at different depths.

The seasonal variability, which can occur as a consequence of the weather conditions and/or of the vineyard treatments, was monitored by considering soils sampled in three different periods of the year, i.e. spring, summer and winter. Therefore, the total number of analyzed samples was 240, namely 16 sampling sites × 5 depths × 3 seasons.

Before the X-ray diffraction analysis, all the samples were crushed using a Teflon spatula, dried at 100 ± 2 °C in an oven for 24 h and then ground by means of a centrifugal mill to a 250 μm particle size. As a matter of fact, homogenized and powdered samples are needed for this analysis.

Finally, the powdered soil samples were stored in hermetic polystyrene containers.

3.3. Experimental

As far as the experimental part is concerned, only a brief description is here presented and the reader should refer to the article reported in Appendix 1 [12] for more details.

However, signal processing exhibits some critical points which are worth discussing further in this section.

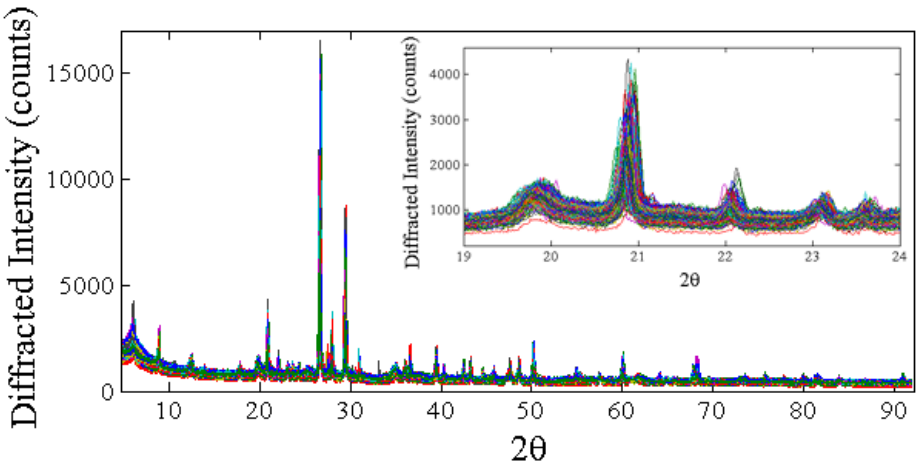

Figure 3.4. Collected diffractograms of soil samples before any pretreatment; the expansion from 19° to 24° 2θ is reported in the square.

All the collected diffractograms were arranged in a data matrix of dimensions 240 × 5200. Because of the complexity of the signals (Figure 3.4), some manipulations were necessary before proceeding with the chemometrics analysis.

The first pretreatment permits the noise reduction and background correction by means of wavelet transform [18, 19], which operates the decomposition and transformation of the signal in a wavelet domain.

Figure 3.5 shows the result of the application of the wavelet transform routine, also including an expansion of the area between 19 and 24° 2θ that highlights the effect of denoising and background correction.

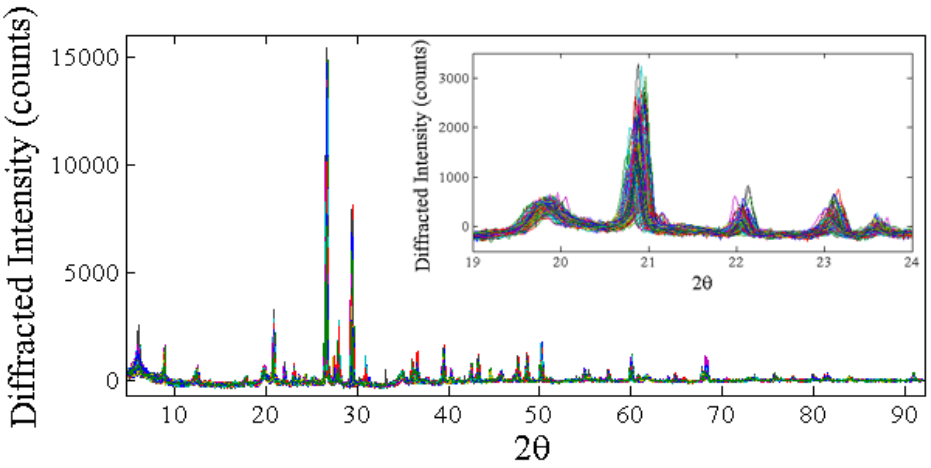

Figure 3.5. XRD signals of soil samples after wavelet transform procedure; the expansion from 19° to 24° 2θ is reported in the square.

Then, signal alignment is needed in order to avoid artificial sources of variability not imputable to real differences among the samples but to measurement procedure. Several causes can produce misalignment, such as, for instance, the manual loading of the samples into the measuring cell and the instrumental drift that can occur from one measuring session to another. The diffractograms of the reference silicon wafer and of the control sample give evidence of these peak shifts.

The soil samples were collected, pretreated (ground, sieved and dried) and analyzed in three distinct periods. In Figure 3.6 are reported the zoom of three raw diffractograms regions for each sampling period (first: red lines; second: blue lines; third: green lines) referring to silica wafer (top, a), control sample (middle, b) and analyzed soil samples (bottom, c). The regions are distributed along all the investigated 2θ interval and are the same for the soil samples and the control samples, namely 8.5 to 9.5°2θ (clays), 26 to 27°2θ (quartz) and 59.5 to 60.5°2θ (quartz); otherwise, it was not possible to select the same regions for the silica wafer, since it is characterized by a reduced number of peaks. Hence, for the reference silica wafer, three different regions were chosen.

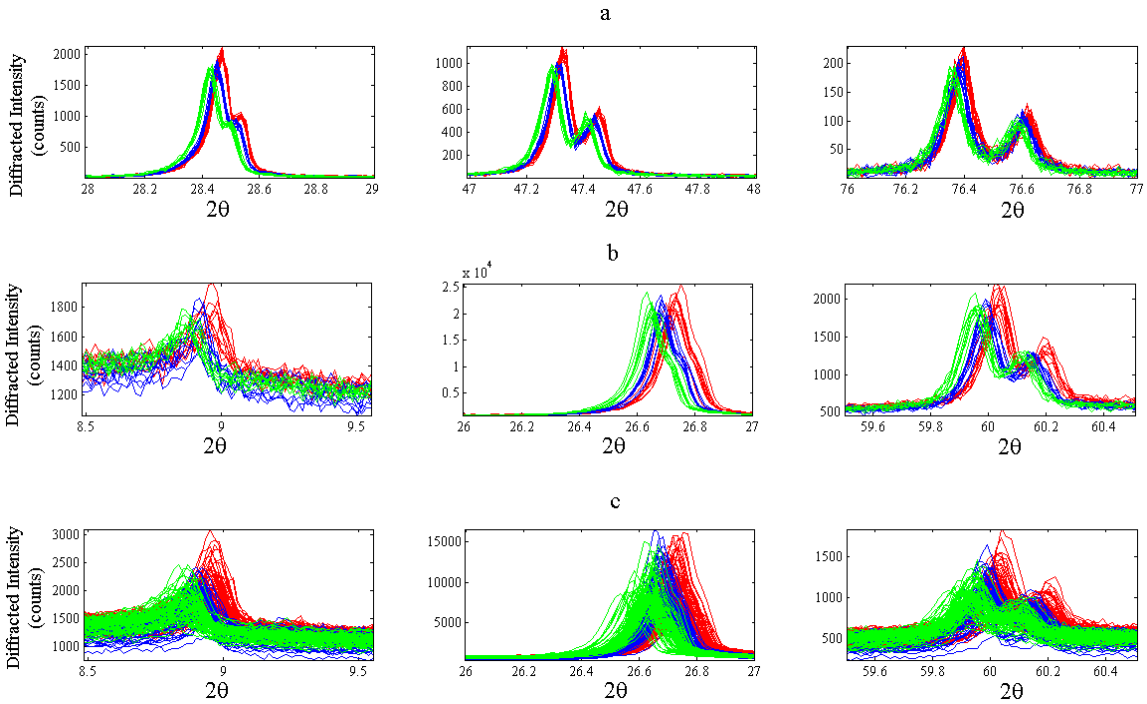

Figure 3.6. Expansion of three regions of the diffractograms referring to silica wafer (a), control sample (b) and analyzed soil samples (c). The diffractograms of each sampling period are reported in different colors (first: red lines; second: blue lines; third: green lines).

It can be noticed that silica wafer horizontal shift among different sessions is almost zero (intra-session) while among different measuring periods is of about 0.05° 2θ; this shift could be attributed to variation of instrumental settings and operative conditions,

since the measured sample is always the same. Control samples intra-session shift is of about 0.05° 2θ and among time periods 0.10-0.15° 2θ.

As far as measured soils samples are concerned the horizontal shift we corrected for is of similar or slightly bigger entity as for the control sample, namely the intra-session shift is of 0.05-0.10° 2θ and those among time periods of 0.10-0.20° 2θ. These shifts remain almost constant at different angle values. Anyhow the algorithm we used for alignment works on windows of the diffractogram and implicitly takes into account non linear shifts with respect to 2θ values.

The spectra alignment was performed by using the *icoshift* algorithm [20, 21] and the result of this pretreatment (with the expansion from 19 to 24° 2θ) is shown in Figure 3.7.

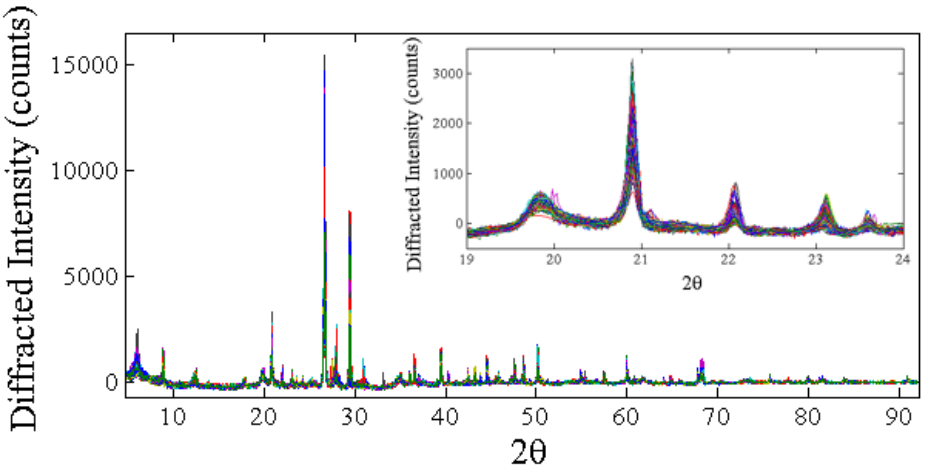

Figure 3.7. XRD signals of soil samples after wavelet transform procedure and alignment by *icoshift*; the expansion from 19° to 24° 2θ is reported in the square.

The resulting signals were then block-scaled [22], so that also the minor peaks can contribute to the model, without altering the relative scale of variables belonging to the same block. Figure 3.8 shows the block-scaled spectra, dotted lines mark the limits of the intervals selected to define each block.

Finally, an exploratory analysis of the data was performed by means of Principal Component Analysis (PCA) [23] on the matrix consisting of 240 samples × 5200 XRD

signal points, whilst Parallel factor analysis (PARAFAC) [24] allows taking into account the three way nature of data set (samples, spectra, depths).

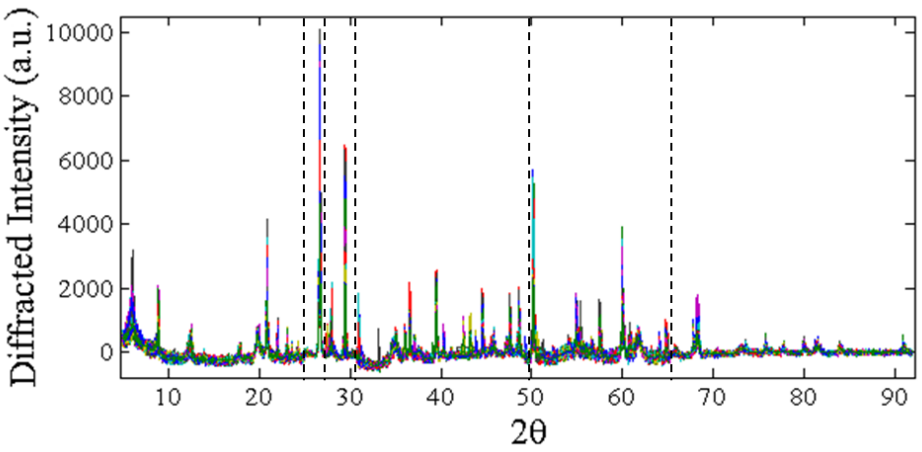

Figure 3.8. Block-scaled XRD signals of soil samples after wavelet transform procedure and alignment by *icoshift*. Dotted lines identify the regions belonging to each group and thus scaled in the same way.

3.4. Results and discussion

Considering the features of our data and the aim of investigating the presence of differences or trends among the soil samples or among the different geographical areas, chemometrics analysis was performed both considering each single sampling period separately, as well as the overall data set.

The results obtained from the analysis of the three partial data sets and the whole one are consistent, therefore the following discussion examines only the latter situation.

3.4.1. PCA analysis of the XRD of soils

The 240×5200 data matrix was firstly analyzed by means of PCA, obtaining a 2 Principal Component (PC) model with explained variance of 75.74%. Most of the

variance is found in the first PC, which mainly accounts for the discrimination among the samples of producer C, all located in the hill region (Figure 3.9).

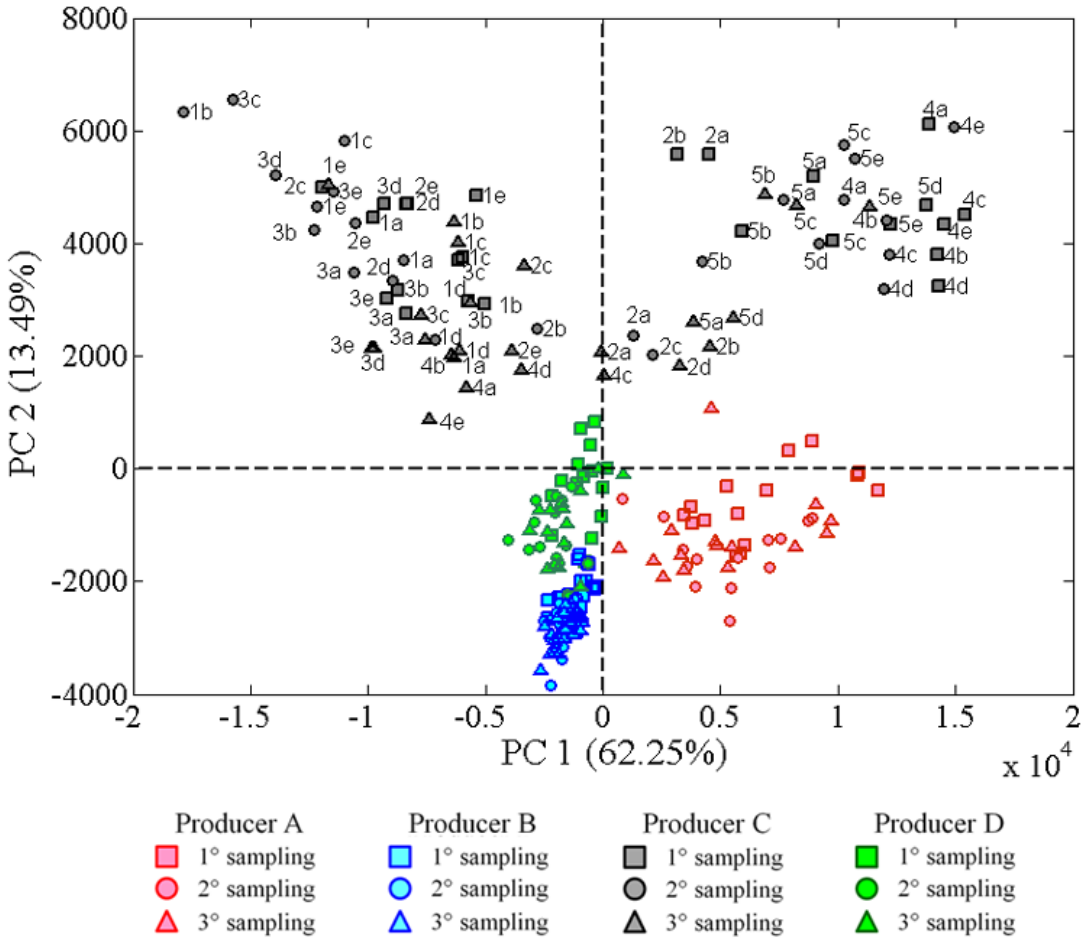

Figure 3.9. PC1 vs. PC2 scores plot for all the collected diffractograms. Colors indicate different producers/fields (red: A, blue: B, gray: C, green: D); symbols indicate the sampling periods (1st sampling, spring: □; 2nd sampling, summer: ○ and 3rd sampling, winter: △). Labels identify the sampling points in the field and the sampling depths, from **a** to **e**.

In particular, the samples coming from point 5, referring to the five sampling depths and the three periods, are placed at positive values of PC1, whilst all the samples of holes 1 and 3 (all depths and periods) are at negative values. As regards point 4, samples collected in the first two periods present positive values, while those of the third period

get mostly negative scores. Differently, soils coming from sampling point 2 are scattered along all the PC1, from negative to positive values. On the contrary, samples located in the in-plain area (producers A, B and D), referring to depths from **a** to **e** and to all the three periods, result grouped in quite compact clusters, which are not perfectly separated and located near the origin of the PC1 axis.

The second PC makes distinction between the hill samples (producer C), with positive PC2 scores, and the in plain soils, located at negative values (producer B) or close to zero (producers A and D). Thus, on the basis of this evidence, it is possible to infer that the second principal component is able to distinguish samples with a different geographical localization.

In order to obtain a better understanding about the diffractogram regions which mainly influence the position of the samples in the scores plot, the loadings plots are reported in Figure 3.10. Further, a basic identification of the phases represented by the diffraction peaks could help recognize the soil features responsible for the sample discrimination.

The lines, representing the loadings values for each component, are colored according to the values of correlation/congruence loadings [25], which allow highlighting the correlation among the original variables and the latent variables resulting from the model. In particular, the closer the correlation/congruence loadings value is to +1 or -1 (red or blue color in Figure 310) the more important the variable is to explain the differences observed among the samples.

The great heterogeneity, which is found for hill samples, is highlighted on the first PC (Figure 3.9) and results to be mainly caused by a different presence of quartz and calcite (Figure 3.10 a). As a matter of fact, the peaks related to these crystalline phases give the main contribution to PC1 loadings. In particular, samples located at positive values of PC1 are characterized by higher amount of quartz and lower of calcite; opposite situation occurs for samples placed at negative values of PC1. Differences in features of the soil samples collected in the hill area were also noticed during the sampling procedure. Moreover, the soil variability of the hill area of Modena district is confirmed in literature [16, 26]. Great complexity, both from a compositional and structural standpoint, is produced by the combination of soils with different characteristics and

geological origins. In particular, soil sparsely calcareous originated hundreds of thousands of years ago from silty-clay sediments of rivers lies close to calcareous soil formed from clay rocks with sandy intercalation of Pliocene age (5-25 million years ago).

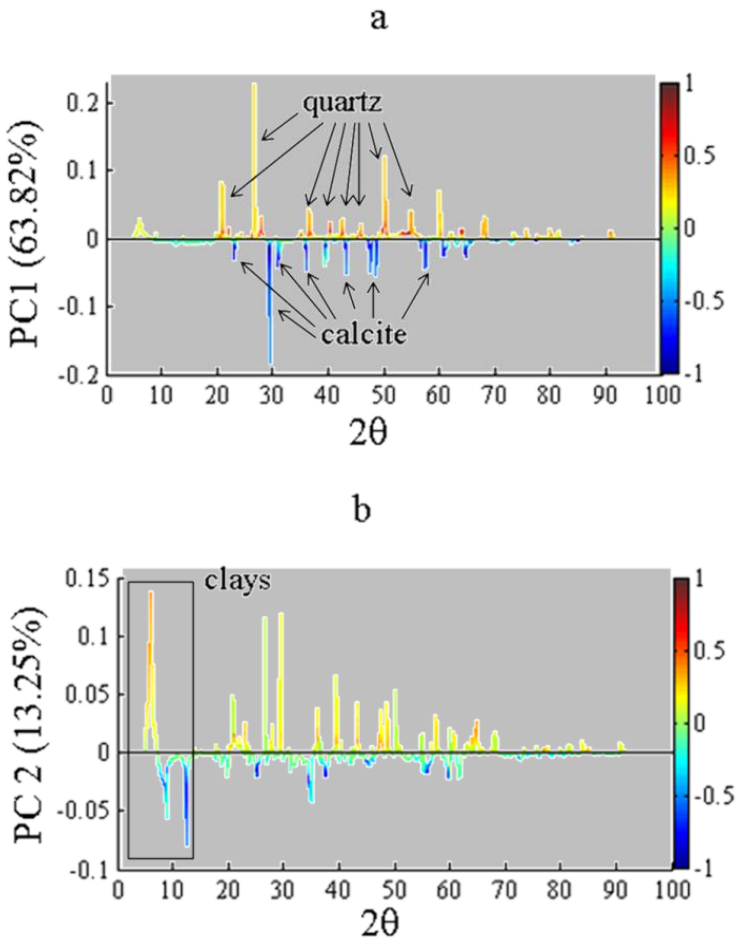

Figure 3.10. PC1 (a) and PC2 (b) loadings plots vs. 2θ values. The loadings profiles are colored according to the values of correlation/congruence loadings. The coding of the color is shown in the color bar.

On the other hand, the second PC accounts for the differences between in-plain and hill samples and is mainly influenced by clay peaks. Notwithstanding the presence of other peaks with high values of loadings, the variation of these phases can be considered

scarcely relevant since the correlation/congruence loadings (represented by the color scale in Figure 3.10 b) tend to zero.

From the results of this elaboration, the seasonal variability is not appreciable, since the samples of the three seasons are almost grouped in a similar way and located in the same position of the scores plot (Figure 3.9). Furthermore, the distances among each sample in the three periods are comparable with those among the repeated measures of a control sample (not reported in the plot). It's worth mentioning that some exceptions for hill samples, in particular those related to point 2 and 4, are found. However, the seasonal variability cannot be completely considered the reason for this behavior, since only few discrepancies were observed. The situation could be probably explained by considering the great complexity and heterogeneity of the hill soils, also associated with not exactly reproducible GPS position of the sampling.

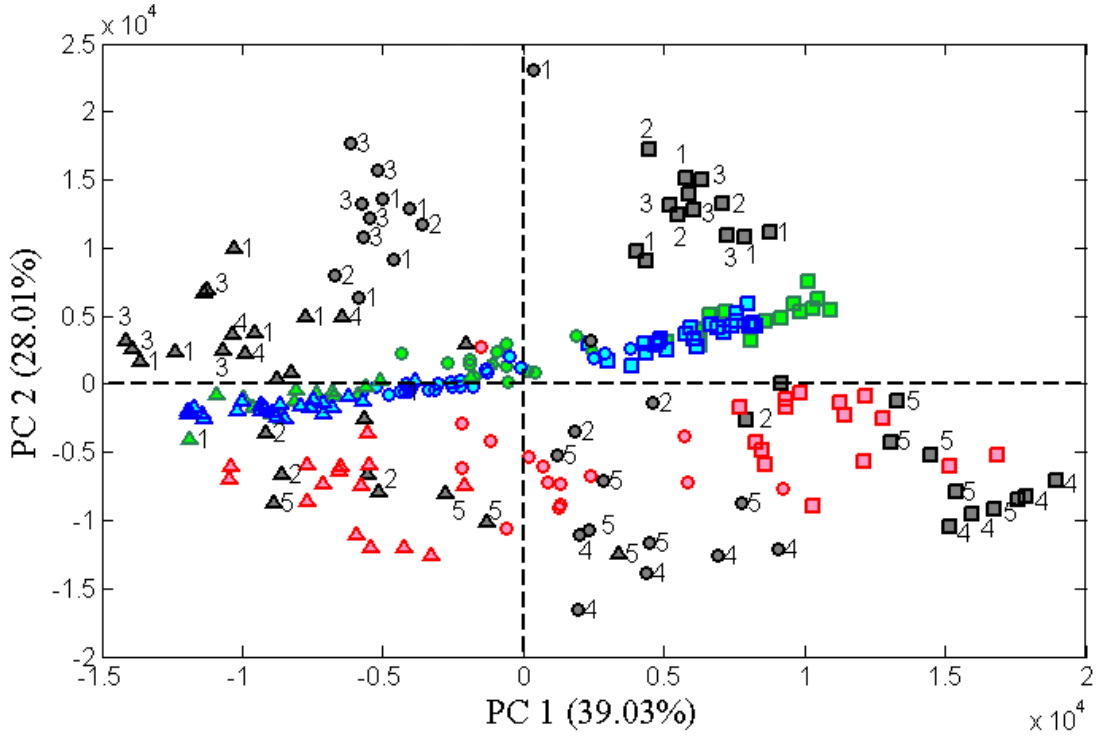

Figure 3.11. PC1 vs. PC2 scores plot for all the non-aligned diffractograms. Colors indicate different producers/fields, whilst symbols refer to the sampling periods. (For better interpretation see Figure 3.9 caption and legend).

Finally, a PCA analysis was performed on the 240×5200 data matrix of the non-aligned diffractograms (4 PCs, explained variance: 82.75%), in order to assess the influence of the alignment procedure on the data and control whether artifacts are introduced by this pretreatment. The chemometrics elaboration reveals that PC1 mainly discriminates the three periods in which samples were collected and measured (Figure 3.11). As a consequence of this source of variability, PC2 vs. PC4 scores plot (Figure 3.12) is required in order to recover the grouping and sampling positioning observed in the previous analysis (Figure 3.9), even if certainly less marked.

Based on these results, the used alignment procedure seems to not introduce artifacts in samples differentiation.

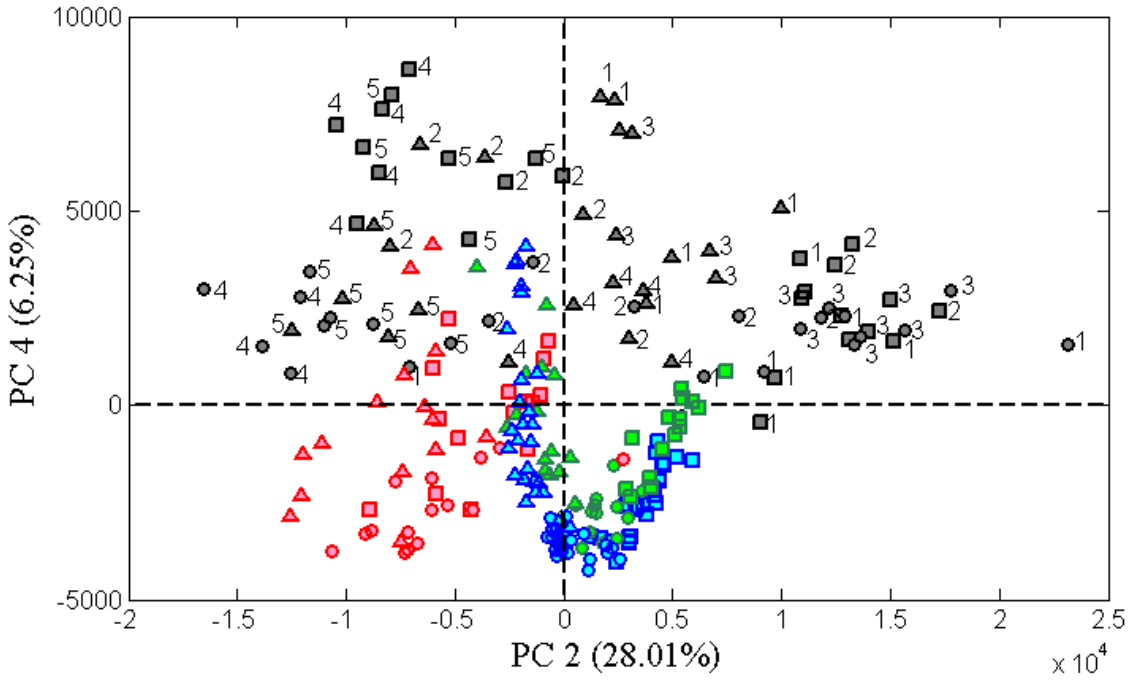

Figure 3.12. PC2 vs. PC4 scores plot for all the non-aligned diffractograms. Colors indicate different producers/fields, whilst symbols refer to the sampling periods. (For better interpretation see Figure 3.9 caption and legend).

3.4.2. PARAFAC model of the XRD of soils

The PARAFAC method was then used in order to take into account the three-way nature of these data, giving relevance to the variability associated to the sampling depths and to the periods when the samples were collected.

The 240×5200 data matrix was rearranged in a 16×5200×5 three-way array with sampling points on Mode 1, X-ray diffractograms on Mode 2 and sampling depths on Mode 3 (Figure 3.13).

Two different centering procedures were compared.

Firstly, centering across Mode 1 was applied, i.e. the data array was unfolded to I × JK and the means from JK columns was eliminated, in order to enhance variability with respect to sampling site (producer/field), by removing the common trend.

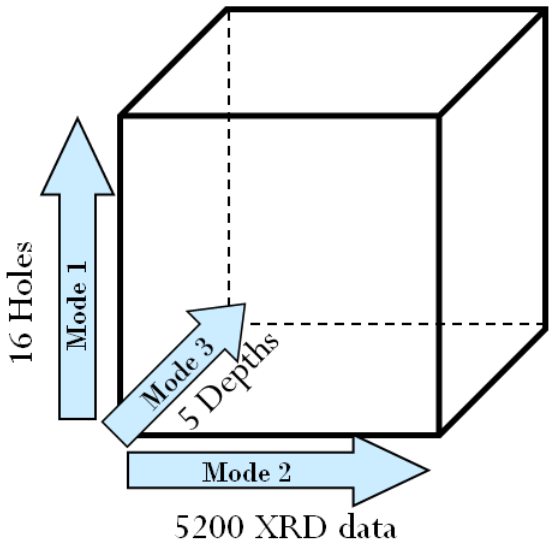

Figure 3.13. Representation of the 3-way data array with sampling points for the 16 sampling periods on Mode 1, the 5200 XRD data points on Mode 2 and the 5 sampling depths on Mode 3.

The resulting 2 Factor (F) model presents explained variance of 69.78% and core consistency of 99%.

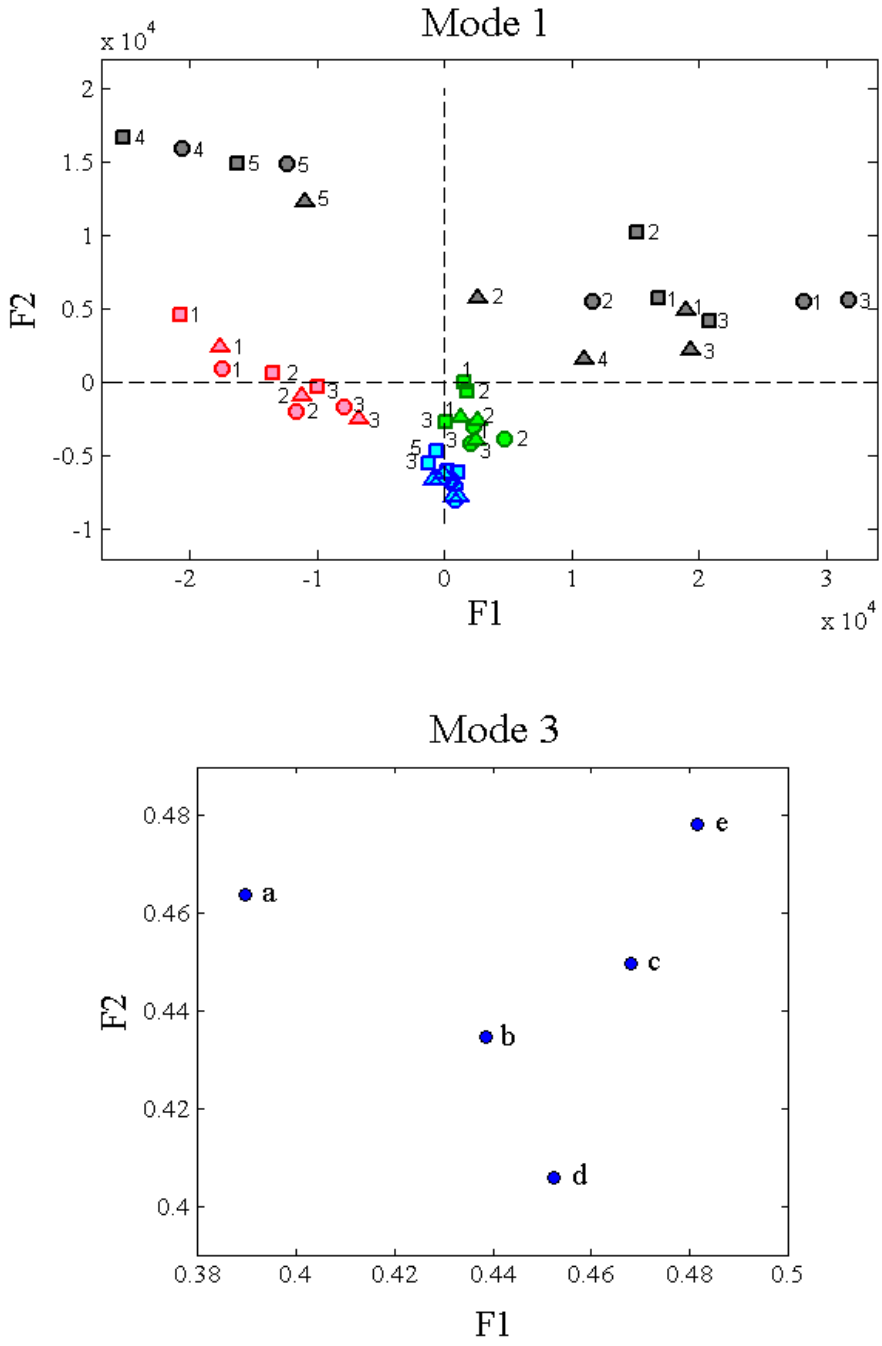

Figure 3.14. F1 vs. F2 PARAFAC scores plot (Mode 1, sampling points) and F1 vs. F2 PARAFAC loadings plot (Mode 3, sampling depths), with centering across Mode 1. In Mode 1 plot, colors indicate different producers/fields (red: A, blue: B, gray: C, green: D); symbols indicate the sampling periods (1st sampling: □; 2nd sampling: ○ and 3rd sampling: △). Labels refer to the sampling points of each field. In Mode 3 plot, labels refer to sampling depths, from **a** to **e**. (For better interpretation see Figure 3.9 legend).

The arrangement of the samples in the Mode 1 scores space (Figure 3.14) is similar to that obtained with PCA analysis, but, in this case, it is more difficult to understand the influence of a single depth on a single sample, since the depths are considered all together for the determination of the position of the samples in Mode 1 scores plot. The Mode 3 loadings plot represents the trend of the depths, considering all the samples, but no common behavior is found.

In order to highlight the differences among the different depths, a centering across Mode 3 was performed, i.e. unfolding the data array to K × IJ and removing means from IJ columns. A model with 2 Factors, explained variance of 23.03% and core consistency of 100% was obtained. The depths result grouped in an upper (**a** and **b**) and a lower (**c**, **d** and **e**) fractions along the first factor in Mode 3 loadings plot (Figure 3.15). However, the arrangement of the samples in Mode 1 plot (Figure 3.15) suggests that the variability within the depth profile is principally influenced by hill samples, in particular sampling points 2 and 5, whilst the other points are located very close to the origin of the axes.

Therefore, only a few number of sampling sites presents differences among the samples collected at different depths, whereas the majority is characterized by a greater "homogeneity" and seems not affected by the sampling depths.

Factor 2 accounts for the variation among the samples collected in the three sampling periods for point 2 of the hill producer. However, the influence of the seasonal variability is not relevant for other samples.

The Mode 2 loadings plots (Figure 3.16) shows that F1 is characterized by calcite peaks at positive values, whilst those of quartz present negative values. As a consequence, the more superficial fractions (**a** and **b**) of point 5 present higher amount of calcite and lower of quartz with respect to the deepest one. Exactly opposite behavior is found for point 2. The seasonal variation occurred for point 2 and highlighted by F2 is mainly due to a varying content of quartz and calcite in the samples collected in different periods.

The present study suggests that sampling depth is negligible for in-plain soils. Consequently, it is reasonable to consider only a sample, averaging the depth profile, for this area, whilst it could be worth to maintain at least an upper (10-30 cm) and a lower (30-60 cm) fractions, as far as hill samples are concerned.

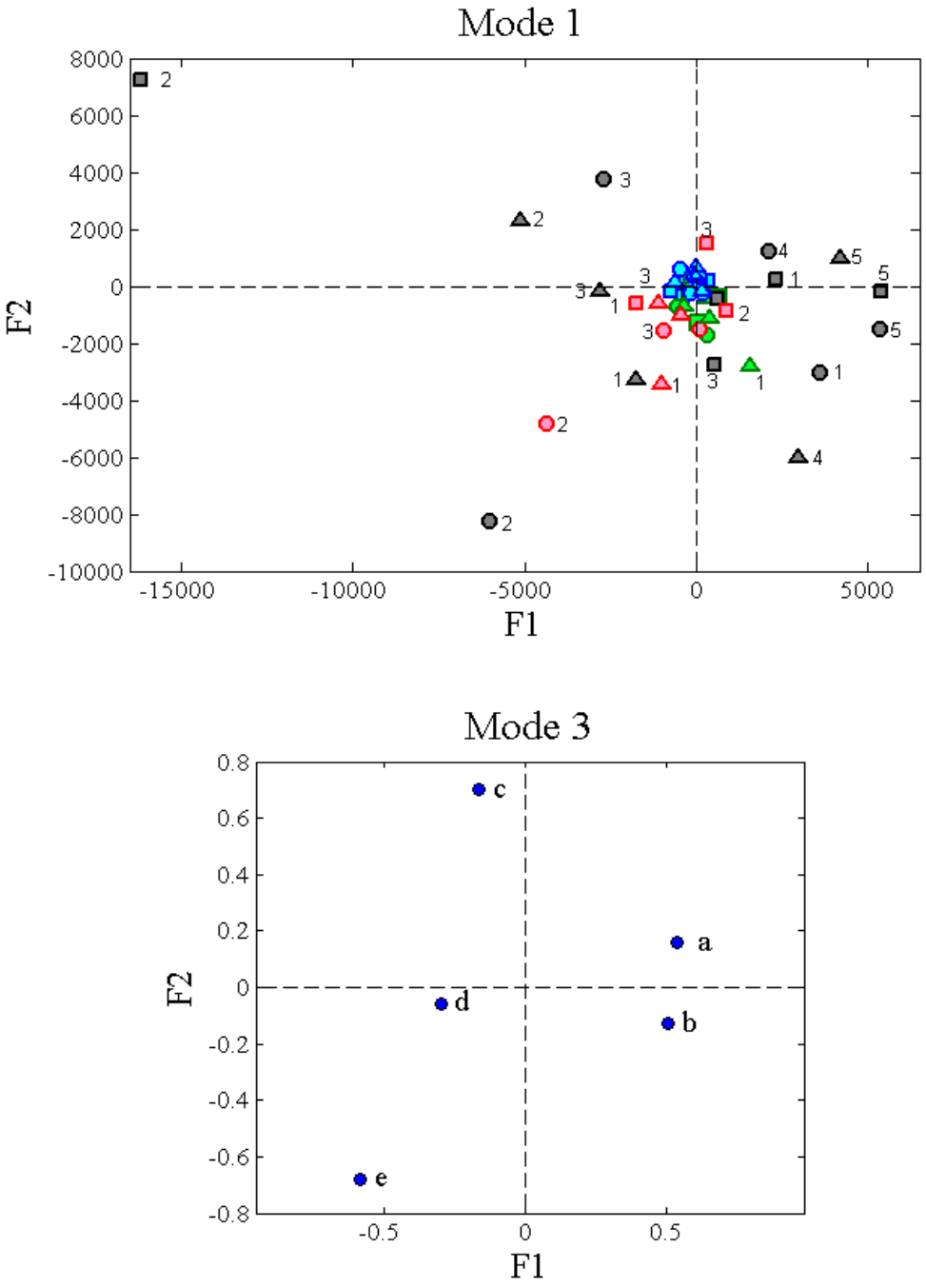

Figure 3.15. F1 vs. F2 PARAFAC scores plot (Mode 1, sampling points) and F1 vs. F2 PARAFAC loadings plot (Mode 3, sampling depths), with centering across Mode 3. In Mode 1 plot, colors indicate different producers/fields (red: A, blue: B, gray: C, green: D); symbols indicate the sampling periods (1st sampling: □; 2nd sampling: ○ and 3rd sampling: △). Labels refer to the sampling points of each field. In Mode 3 plot, labels refer to sampling depths, from a to e. (For better interpretation see Figure 3.9 legend).

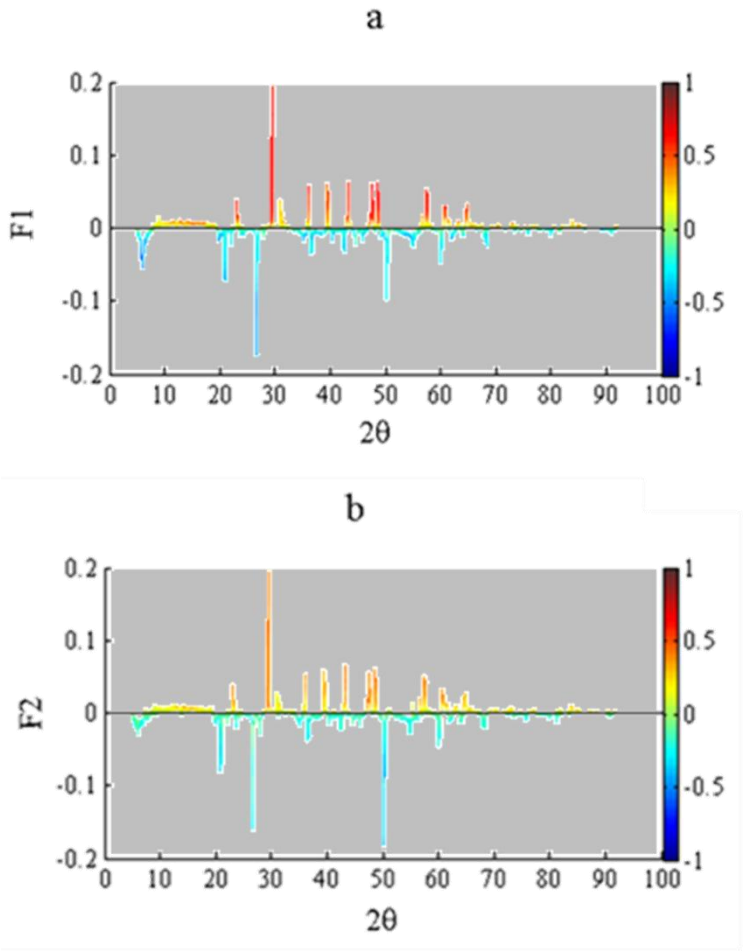

Figure 3.16. PARAFAC (model obtained with centering across Mode 3) loadings plots (Mode 2, XRD signals) vs. 2θ values: (a) F1 and (b) F2. The loadings profiles are colored according to the values of correlation/congruence loadings. The coding of the color is shown in the color bar.

3.4.3. Comparison with other analytical techniques

The results obtained by other analytical techniques were considered, with the aim of confirming the potentiality of the X-ray diffraction analysis coupled with chemometrics techniques for the assessment of soil variability and of supporting the previous considerations.

At first, a parallel study evaluated the metals content on the HNO_3 extractable fraction from the same soil samples limited to the first sampling period. All the sampled depths

were considered for the hill producer (C), whilst only the **a** and **b** fractions were analyzed for the in plain ones (A, B and D). Na and K were determined by means of FAAS, Ca and Mg by ICP-OES and V, Cr, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Cd, Cs, Ba, La, Ce, Pr, Nd, Sm, Eu, Gd, Dy, Ho, Er, Tm, Yb, Lu, Tl, Pb, Th and U using ICP-qMS. The PCA model (2PCs, explained variance: 70.12%) built for the autoscaled data matrix reveals an almost identical discrimination of the producer sites and sampling depths (Figure 3.17). In particular, hill samples (colored in gray) result again different from the in-plain ones and characterized by a great heterogeneity, as regards both the sampling points and depths. The other samples, even if not perfectly separated, present higher homogeneity.

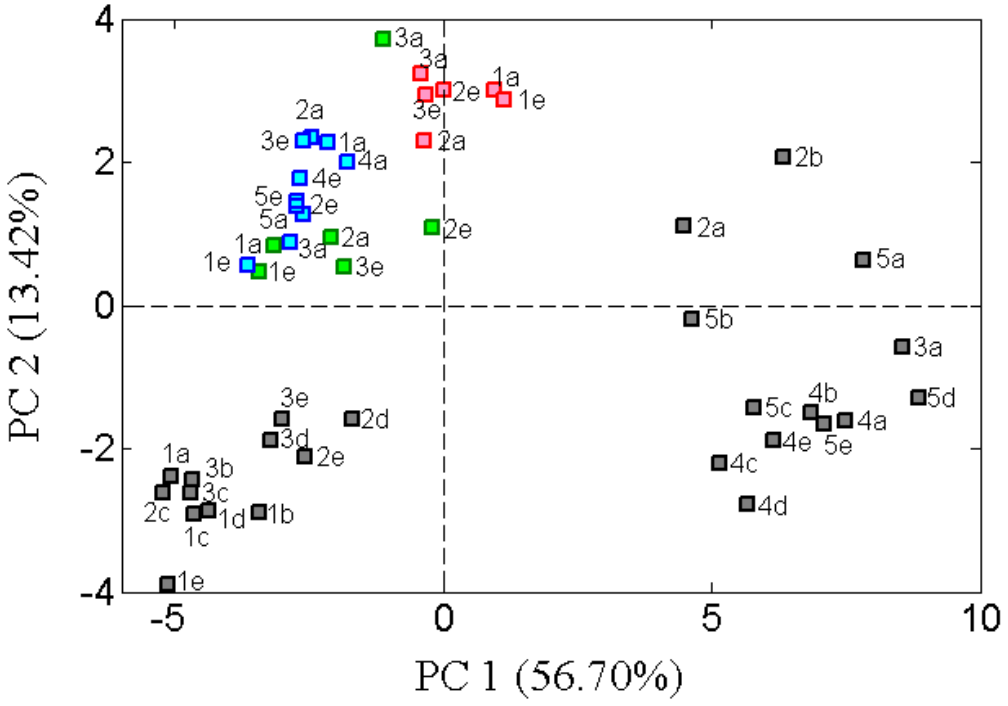

Figure 3.17. PC1 vs. PC2 scores plot for the analyzed soil samples. Colors indicate different producers/fields (red: A, blue: B, gray: C, green: D); labels identify the sampling points in the field and the sampling depths, from a to e. (For better interpretation see Figure 3.9 legend).

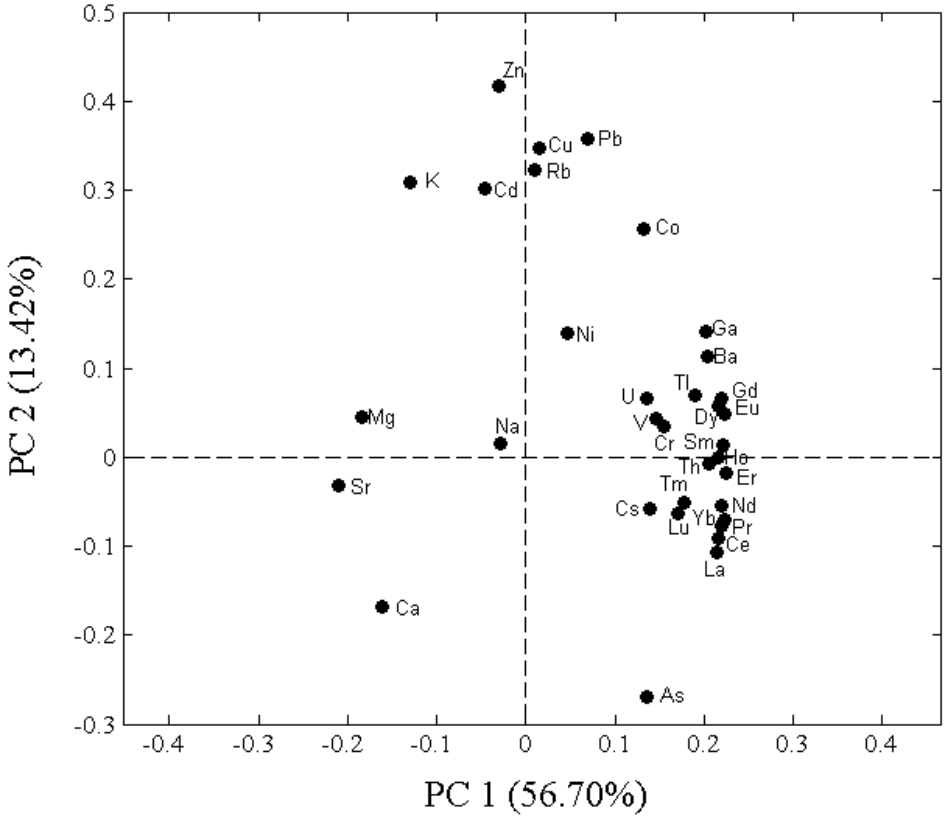

Figure 3.18. PC1 vs. PC2 loadings plot for the metal content measured on the HNO_3 extractable fraction of soils.

As regards hill samples, the loadings plot (Figure 3.18) shows that points 4 and 5 and the upper fractions (**a** and **b**) of point 2 are characterized by higher content of rare earth elements (REEs), being positioned at positive values of PC1. The remaining hill samples, located at negative values of both PC1 and PC2, present higher concentration of Ca and Sr. On the other hand, in-plain soils are richer in K, Zn Rb, Cu and Pb.

Further, the same pattern in soil samples differentiation was also observed from the PCA analysis of other available data, obtained by different research groups for the soil samples of the first and second sampling periods and here reported for comparison purposes. In this case, all the depths were considered for the hill producer, whilst only **b** and **e** fractions were analyzed for the in-plain ones.

The considered variables are:

- SiO_2, TiO_2, Al_2O_3, FeO, MnO, MgO, CaO, K_2O, Na_2O, P_2O_5, Ce, Nd, Sr, Rb, Pb, Zn content, determined by means of X-Ray Fluorescence (XRF);
- Loss on ignition (P.F.) and $CaCO_3$ by means of carbonate-content analysis (calcimetry);
- $\delta^{13}C$ (‰) and $\delta^{15}N$ (‰) by means of Isotope Ratio Mass Spectrometry (IRMS);
- $^{87}Sr/^{86}Sr$ isotope ratio (Sr IR) by means of MC-ICP-MS.

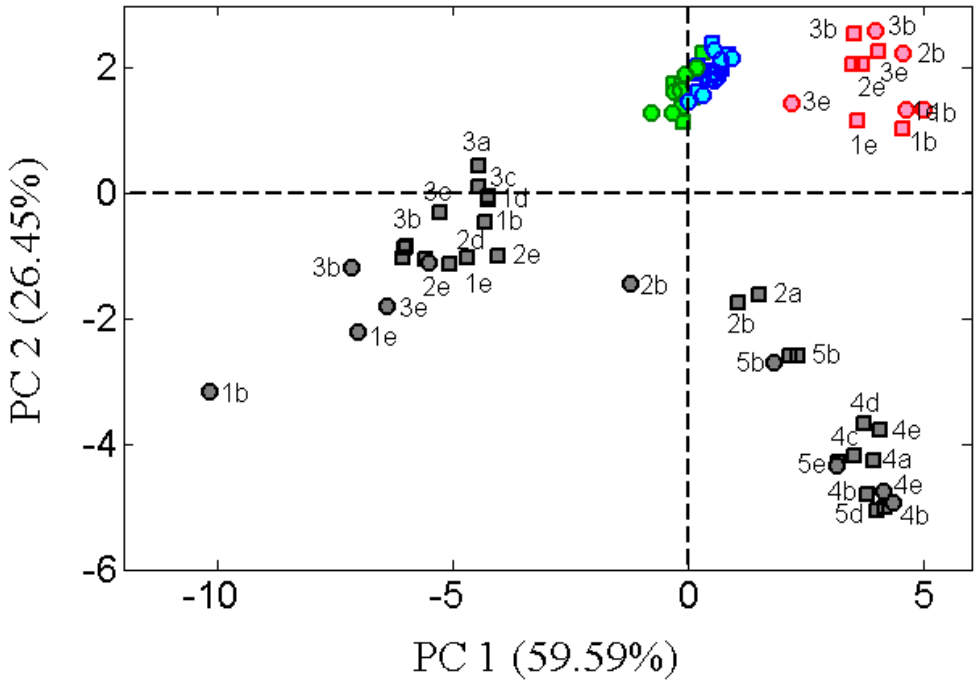

Figure 3.19. PC1 vs. PC2 scores plot for the analyzed soil samples. Colors indicate different producers/fields (red: A, blue: B, gray: C, green: D); symbols indicate the sampling periods (1st sampling: □; 2nd sampling: ○). Labels identify the sampling points in the field and the sampling depths, from a to e. (For better interpretation see Figure 3.9 legend).

The 2 PCs model obtained by the autoscaled data matrix has explained variance of 86.04%. As previously mentioned, the results (Figure 3.19 and Figure 3.20) are

consistent with those of the XRD analysis (Figure 3.9 and Figure 3.10) as well as with those regarding the metal content (Figure 3.17 and Figure 3.18). From a comprehensive analysis, hill and in-plain samples show very different features both from a chemical and a structural point of view, probably because of their different genesis. This leads also to different $^{87}Sr/^{86}Sr$ values, higher for the hill samples with respect to the in-plain ones, being Sr IR variable negative on the PC2 (Figure 3.20).

The separation of hill samples is probably due to the union of two different hill slopes characterized by a macroscopic calcareous vein, visible to the naked eye, which passes through the field dividing it in two parts. This takes also evidence from the higher amount of CaO (from XRF), $CaCO_3$ (from calcimetry), Ca (from ICP-OES) and calcite (from XRD) in the holes 1 and 3 and in the lower fractions of hole 2.

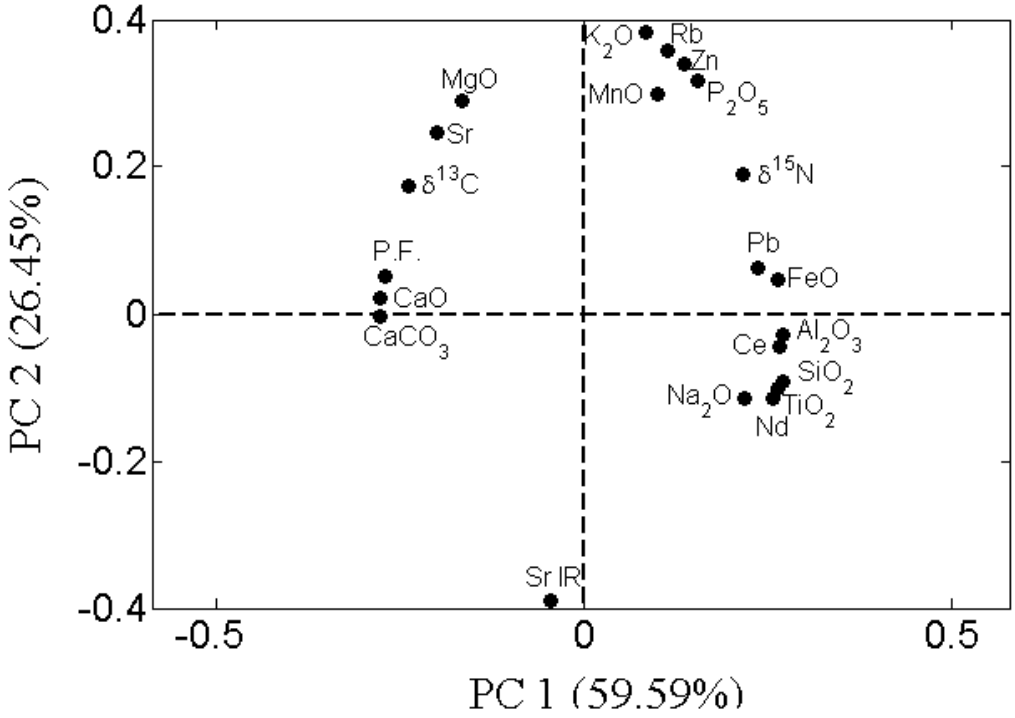

Figure 3.20. PC1 vs. PC2 loadings plot for the 21 considered variables.

3.5. Conclusions

The soil sampling procedure represents an essential starting point for any geographical traceability study. X-ray diffraction analysis and chemometrics techniques gave great support, since this approach allowed investigating soil variability, by considering the inter- and intra-site variations, the depth profile and seasonal influence.

This preliminary study regards soil samples collected both on in-plane and hill areas of the province of Modena. Firstly, the presented approach allowed obtaining a basic knowledge of the investigating area, as regards structural and compositional features.

The analyses present hill samples well distinct from the in-plains ones and characterized by a large horizontal variability and somewhat also along the sampling depth profile. On the other hand, the in-plain areas resulted more homogeneous even in the vertical profile. As regards the seasonal variability, the choice of the sampling period does not seem to have "significant influence" on the obtained results.

This screening of the province of Modena provided some practical indications to guide the planning of soil sampling procedures. In particular, the choice of the number of soil samples and their type (i.e. single soil carrot or fractionated ones, split in upper and lower parts) is of fundamental importance for the extensive sampling on the whole Modena district and the selection of a reduced number of samples on which perform a more detailed investigation (i.e. determination of the traceability indicators).

Indeed, on the basis of these results, the separation of the soil carrot in 5 depth fractions could be avoided for the in-plain area, merging all the aliquots or at least maintaining 2 samples (an upper fraction and a lower one) for the preliminary investigations. Owing to the reduction of the number of samples to be processed, an increased number of points could be sampled, allowing a deeper exploration of the horizontal variability.

As far as the hill samples are concerned, a greater heterogeneity is revealed, thus suggesting a careful study of this area both along the horizontal and the vertical directions.

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CHAPTER 4

Strontium isotope ratio: from soil to grapes

4.1. Introduction

When dealing with geographical traceability of oenological products, one of the main key points is to be sure about the robustness of the developed models, strongly associated with the potentiality of the investigated tracer, which directly links the rock/soil to the respective final product.

Among the direct indicators used for this purpose, the isotopic pattern of strontium (Sr), and in particular the $^{87}Sr/^{86}Sr$ isotope ratio (Sr-I.R.), shows promising perspectives in different areas, mainly in food traceability studies (see Section 2.3). As geographical tracer for food, one of its important features is that the element can be easily assimilated by plant's roots as substitute for calcium, keeping its fingerprint unchanged, i.e. the isotope abundances do not undergo to appreciable fractionation [1].

However, as described in Chapter 2, different phenomena could influence the value of Sr-I.R. found in the investigated food, namely grape juice, which could be different from that obtained for the rock, as well as for the soil of provenance.

On the basis of these considerations, two important issues have to be taken into account for developing trustworthy geographical traceability models: the representativeness of food as well as soil sampling (number of samples, sampling procedures and so on) and the variability of the investigated parameter within the studied soil-grapevine system.

As regards the first issue, an *a priori* knowledge of the chemical and geological features of the territory is needed in order to select the appropriate sampling location and procedures as well as a number of samples statistically representative of the investigated area (see Chapter 3 and [2]).

As far as the variability of $^{87}Sr/^{86}Sr$ from soil to food is concerned, as widely described in Chapter 2, a limiting factor for the development of reliable traceability models lies in the discrepancies found between the $^{87}Sr/^{86}Sr$ values of the soil and those of the biologically available fraction, i.e. the elements part the plant is able to absorb from the soil.

Considering the aim of the research, it is clear that the evaluation of the more representative indicator of the grapevine system can be obtained if it is performed on

the elements fraction which better accounts for the plant uptake. However, this fraction is highly dependent not only on the element concentrations in the soil, but also on its geomorphologic features, clay content, pH, organic matter and Cation Exchange Capacity (CEC) [3]. Thus, its determination is quite complex and different studies have been carried out in order to find a correlation between the element concentration in the fraction extracted with various chemical media and that determined in some parts of model plants [3–10]. Several analytical methodologies have been investigated, using different extraction media. In particular, NH_4NO_3 1 M, $CaCl_2$ 10 mM, NH_4Cl 1 M, $Na_4P_2O_7$ 0.1 M, KNO_3 1 M, H_2O, $NaNO_3$ 0.1 M and NH_4Ac 1 M are classified as mild extractants; HNO_3 0.1 M and EDTA 0.05 M, as strong extractants. Comparative studies have shown that mild extractants better simulate the uptake of the model plant, whereas the strong ones generally overestimate it. Among the mild media, H_2O is usually not chosen, since it is too weak and, in the water extract, colloidal systems are present, negatively influencing the procedure and the following determinations. The more used chemical medium is ammonium nitrate (NH_4NO_3), since it has low reactivity and does not deeply change the pH, thus producing similar conditions to those of the soil-root system [6]. Indeed, NH_4NO_3 leads to a slight pH decrease and an increase in the ionic strength, causing the precipitation of colloids and metal-organic complexes. Moreover, owing to the ionic strength conditions, the activity of the metal-OH^+ species decreases, thus helping the metal ions desorption from the negatively charged surfaces of the soil constituents [7]. NH_4Cl presents similar features, but produces a large amount of Cl^- ions, which are interferences in the following separation procedure and ICP-MS determination.

In the cases here presented, the $^{87}Sr/^{86}Sr$ isotope ratio for soils sampled at different depths and in various locations was measured on the bio-available fraction obtained by extraction with NH_4NO_3, according to the DIN 19730 standard procedure [11]. Furthermore, a comparison between these values and those obtained with another extraction procedure, namely HNO_3 digestion, was illustrated, in order to assess the presence of a deviation in the results, due to different forms in which the Sr is found in the soil. Then, the indicator was also determined on grape juices and the values were

compared with those obtained for the respective soils. The aim of this study is to establish how and if the measured Sr-I.R. varies according to the geological stratification of soils and to determine the best correlation between soil and food, assessing whether the extracted soil fraction is able to mimic the grapevine uptake and thus having a more defined picture of the overall potentialities of the indicator.

Finally, considering the previous statements about the difficulties arising in the soil sampling and in the evaluation of the raw plant uptake, a conversion in the sampling approach for traceability purposes is suggested. A "passive" strategy, which assumes to collect soil powders with the related difficulty, could be supported or substituted by an active one, where the plants directly do the sampling.

The plants, indeed, have direct access to the bio-available element reservoir of the soil, also extending the sampling uptake to the neighbor area of the growing vine. Moreover, the measured values take into account the influence of all the processes occurring in the environment, such as weathering, aerosol uptake, water contribution, pollution agents and contamination due to the use of fertilizers.

This approach allows getting around the problems related to the soil sampling and analysis, as regards both the sample location and sampling procedures as well as the choice of the suitable chemical agent for the extraction of the bio-available fraction.

In this Chapter, the above mentioned aspects are handled with reference to the case of three different Italian wines.

The first one regards the *Lambrusco* wines, which are PDO products typical of the Modena province, whose production regulations [12–14] allow the grape cultivation in the whole district. This area is estimated to be about 2700 km^2 of which 90 km^2 are grape cultivated, ranging from in-plane to hill territories. Because of the extension of the considered territory, relatively "large scale" models are needed. So, this investigation results to be a pilot study aimed to obtain more information, before proceeding with an extensive mapping.

The other two investigated products are *Chianti Classico DOCG* wines [15] and *Barolo DOCG* wines [16]. These cases of study are different from *Lambrusco* wine one, since the latter disciplinary regulations involve "more restricted" areas, as for the *Chianti*

Classico wines, or the quality/reputation is linked to the producer's brand (e.g. *Barolo* wines). Therefore, more detailed or tailored traceability models are required, i.e. based on a more punctual sampling.

4.2. Sampling and samples

4.2.1. *Lambrusco PDO* wine case of study

For the *Lambrusco PDO* case of study, four farms (A, B, C and D) of the Modena district characterized by a "long production chain" (from grape to bottled wine) were considered, as described in Section 3.2. Three to five sampling points were selected within each field, giving a total number of sixteen sampling points (see Figure 3.2). As regards soil, five aliquots at different depths were sampled for each point (see Figure 3.3) and the sampling procedure was repeated in three different periods of the year. Nevertheless, on the basis of the results reported in Chapter 3, $^{87}Sr/^{86}Sr$ isotope ratio was determined only for **a** and **e** depth fractions. Furthermore, a deeper investigation of the hill producer/field C was performed by considering all the soil depths coming from the first sampling period.

From each field, vine branches and grape juices samples were collected from the grapevines grown in proximity of the sampled holes in the soil. Branches were picked up during the first and second sampling procedures, whilst grapes refers to the production of the following year, thus giving a total of thirty-two samples for the first matrix type and sixteen for the second one.

4.2.2. *Chianti Classico DOCG* wine case of study

Soil samples were collected in two different fields of a producer located in the *Chianti Classico* production area in Tuscany, as shown in Figure 4.1. The GPS positions of the sampling points are reported in Table 4.1. Soil holes were dug by a single gauge auger

set for hardly disturbed samples at a maximum sampling depth of roughly 70 cm. The obtained carrots were divided in two aliquots (**up** and **down**) of 30 cm length each, discarding the upper 10 cm.

During the soil sampling procedure, vine branches were picked up too. In particular, some logs, 10 to 20 cm long, were cut from the producing part of the plants growing close to the seven sampling points.

Figure 4.1. Sampling points for the two fields of the *Chianti Classico* producer.

Table 4.1. GPS position (expressed in degree, minutes, and seconds) of the sampling points of the *Chianti Classico* producer.

First field		Second field	
1I01	N 43° 30' 27.32" E 11° 14' 04.28"	**2I03**	N 43° 30' 57.91" E 11° 13' 13.14"
1I02	N 43° 30' 28.26" E 11° 14' 04.77"	**2I04**	N 43° 31' 01.47" E 11° 13' 14.32"
		2I05	N 43° 31' 03.25" E 11° 13' 13.94"
		2I06	N 43° 31' 02.70" E 11° 13' 07.54"
		2I07	N 43° 30' 59.62" E 11° 13' 07.96"

Finally, two samples of intermediate product (hereafter called pre-wines) were collected some months after the grape harvest, after the alcoholic and malo-lactic fermentations, but not yet aged. PreW1I is the pre-wine sample relative to the grapes coming from the first field, whilst PreW2I refers to the second field's grapes.

4.2.3. *Barolo DOCG* wine case of study

Soil samples were collected from two different producers located in the *Barolo* production area in Piedmont. As regards the first producer, samples were collected from one field (Figure 4.2) and the GPS positions of the eleven sampling points are reported in Table 4.2.

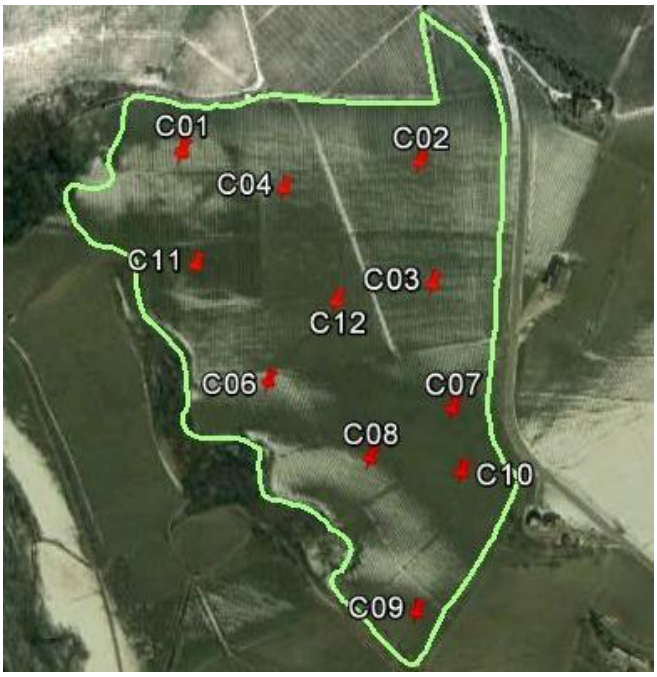

Figure 4.2. Sampling points for the first *Barolo* producer.

Table 4.2. GPS position (expressed in degree, minutes, and seconds) of the sampling points of the first *Barolo* producer.

First *Barolo* producer			
C01	N 44° 35' 30.70" E 07° 59' 58.03"	**C08**	N 44° 35' 22.10" E 08° 00' 05.53"
C02	N 44° 35' 30.30" E 08° 00' 07.41"	**C09**	N 44° 35' 17.75" E 08° 00' 07.38"
C03	N 44° 35' 26.93" E 08° 00' 07.90"	**C10**	N 44° 35' 21.75" E 08° 00' 09.04"
C04	N 44° 35' 29.67" E 08° 00' 02.14"	**C11**	N 44° 35' 27.55" E 07° 59' 58.58"
C06	N 44° 35' 24.22" E 08° 00' 01.52"	**C12**	N 44° 35' 26.48" E 08° 00' 04.22"
C07	N 44° 35' 23.51" E 08° 00' 08.66"		

Figure 4.3. Sampling points for the two fields of the second *Barolo* producer.

Table 4.3. GPS position (expressed in degree, minutes, and seconds) of the sampling points of the second *Barolo* producer.

Second *Barolo* producer			
First field		**Second field**	
1M01	N 44° 36' 27.77"	**2M01**	N 44° 37' 35.86"
	E 07° 58' 22.57"		E 07° 58' 12.23"
1M02	N 44° 36' 25.52"	**2M02**	N 44° 37' 34.06"
	E 07° 58' 18.58"		E 07° 58' 13.02"
1M03	N 44° 36' 24.04"	**2M03**	N 44° 37' 31.40"
	E 07° 58' 17.45"		E 07° 58' 17.34"
1M04	N 44° 36' 24.58"	**2M04**	N 44° 37' 29.56"
	E 07° 58' 19.25"		E 07° 58' 16.81"
1M06	N 44° 36' 32.61"	**2M05**	N 44° 37' 30.44"
	E 07° 58' 10.48"		E 07° 58' 20.27"
		2M06	N 44° 37' 28.58"
			E 07° 58' 21.25"
		2M07	N 44° 37' 27.55"
			E 07° 58' 25.45"
		2M08	N 44° 37' 28.76"
			E 07° 58' 27.22"
		2M09	N 44° 37' 29.80"
			E 07° 58' 24.92"
		2M10	N 44° 37' 32.56"
			E 07° 58' 14.49"

As regards the second producer, samples were collected from two different fields (Figure 4.3) and the GPS positions of the fifteen sampling points are reported in Table 4.3. Similarly to the *Chianti Classico* case, soil was sampled from each point, obtaining one **up** fraction (from 10 to 40 cm) and one **down** fraction (from 40 to 70 cm).

Four samples of intermediate product (hereafter called pre-wines), relative to the grapes coming from whole field of the first producer, were collected, after the alcoholic and malo-lactic fermentations, but not yet aged. PreW1C1 is a sample which is becoming *Barbera d'Alba*, whilst PreW1C2, PreW1C3 and PreW1C4 are *Barolo* pre-wines.
As regards the second producer, six pre-wine samples (not yet aged) were collected. In particular, PreW1M is relative to the grapes coming from the first field (sampling points 1M01,1M02, 1M03, 1M04 and 1M06); PreW2M1 refers to grapes coming from the area close to sampling points 2M07, 2M08, 2M09 and 2M10; PreW2M2 to points 2M01 and 2M02; PreW2M4 to point 2M05; PreW2M5 to points 2M03, 2M04 and 2M06; PreW2M3 is made by 2/3 of grapes located near point 2M10 and 2/3 near point 2M05.

4.3. Experimental

4.3.1. Pretreatment and extraction procedure of soil samples

In order to obtain homogeneous and powdered samples, soils have to be properly pretreated. Each soil sample was minced using a Teflon spatula and then dried at 100 ± 2 °C in an oven for 24h. Then, the soils were ground by using a centrifugal mill (refer to Appendix 1) to a 250 μm particle size and finally stored in polystyrene (PS) containers, at room temperature.
The determination of the bio-available Sr fraction in soil was performed by the extraction procedure DIN 19730 [11] with 1 mol L^{-1} NH_4NO_3 solution. The obtained eluate was centrifuged at 3000 rpm for 10 minutes and then filtered through cellulose acetate membrane (pore size = 0.20 μm) into a 30 mL PFA bottle.
The extraction with HNO_3 was carried out by means of an optimized microwave (MW) assisted procedure [17]. Briefly, soil leaching was performed on a maximum sample aliquot of 0.5 g accurately weighted into the MW vessel, then added with 10 mL Ultrapure 65% HNO_3. Each mineralization cycle was set as shown in Table 4.4 and the vessel carousel was loaded with 6 soil samples, 1 process blank (10 mL of 65% HNO_3)

and 1 control sample (soil sample). At the end of the extraction process, each solution was filtered with cellulose acetate membrane (pore size = 0.20 μm) into a 30 mL PFA bottle. Between each cycle of mineralization, a washing cycle (Table 4.5) was always performed.

Table 4.4. Microwave program used for the acid extraction of soil samples

	Power (watt)	**Power (%)**	**Ramp (min)**	**Temperature (°C)**	**Time @ Temp (min)**
1 step	800	100	5:00	80	1:00
2 step	800	100	10:00	169	24:00

Table 4.5. Microwave operating condition used for the washing cycle after soil matrix extraction. Washing cycle was carried out using 10 mL 65% HNO_3

	Power (watt)	**Power (%)**	**Ramp (min)**	**Temperature (°C)**	**Time @ Temp (min)**
1 step	800	100	10:00	150	15:00

For each sample, the strontium concentration was determined in order to dilute the solution for working within the optimal instrumental conditions in terms of accuracy and precision of the measurements.

In particular, for the Sr/Rb separation procedure, the maximum retention capability is achieved when working with 8 M nitric acid solution and, after this step, the best instrumental precision for Sr-I.R. measurement is obtained with Sr concentrations close to 200 μg kg^{-1}.

4.3.2. Digestion procedure of vine branches, grape juices and wines

After the sampling, branch samples were cut in 1cm long pieces, dried at 105 °C for 24h and ground with the same centrifugal mill used for the soils.

Grape juices and vine branches digestion was performed on a maximum sample aliquot of 4 g and 0.3 g, respectively, accurately weighted into the vessels, then added with 6 mL HNO_3, 1 mL H_2O_2 and 3 mL H_2O. The procedure is described in [18].

As regards wines, the digestion was performed by adding 5 mL of 65% HNO_3 to 5 mL of wine sample. This mixture is left to react in a vessel for 12h at atmospheric pressure and room temperature, as illustrated in [19].

At the end of the digestion process, all the solutions were transferred in PFA bottles and the Sr content was determined in order to dilute the obtained sample according to the suitable properties, as described for soil samples.

4.3.3. Interference separation

The $^{87}Sr/^{86}Sr$ determination is mainly influenced by the presence of the isobaric interference of ^{87}Rb. This interference is usually minimized through a matrix simplification procedure, which generally involves a SPE (Solid Phase Extraction) separation whose specificity and selectivity for strontium are pH dependent [20]. The reliability of the separation procedure is extremely important to achieve precise and accurate measurement of the isotope ratio.

The setting parameters of the soils separation were optimized by means of Design of Experiment (DoE) approach [17, 21] and the procedure could be summarized as follows: i) 2 mL of resin suspension are loaded into the SPE column; ii) the resin is washed with 2 mL of high-purity water and activated with 12 mL of 8M HNO_3; iii) 10 mL of sample in 8M HNO_3 are loaded; iv) the interferences are eluted with 4 mL of 8M HNO_3 and v) the recovery of Sr is accomplished by using 12 mL of high-purity water.

Also the Sr/Rb separation for organic matrices (branches, juices, wines) was optimized by means of DoE [19] and basically consists of the following steps: i) 1 mL of resin suspension is loaded into the SPE column; ii) the resin is washed with 2 mL of high-purity water and activated with 5.5 mL of 8M HNO_3; iii) 10 mL of sample in 8M HNO_3 are loaded; iv) the interferences are eluted with 3.5 mL of 8M HNO_3 and v) the recovery of Sr is accomplished by using 7.5 mL of high-purity water.

At the end of the separation procedure, a proper aliquot of 65% HNO_3 was added to all the water eluted fractions to obtain 4% HNO_3 final solutions.

4.3.4. Reagents and materials

All the sample preparation procedures were carried out under horizontal laminar flow hood equipped with an HEPA filter, in order to prevent the occurrence of any ambient contamination. Solutions were prepared by using high-purity deionized water TYPE1 (physical and chemical parameters for TYPE1 water comply with ASTM TYPE I and ISO3696 GRADE I purity specifications) obtained from a Milli-Q system (Millipore, Bedford MD) with a resistivity better than 18 MΩcm. Ultrapure HNO_3 65% w/w was obtained from analytical grade nitric acid (Carlo Erba, Milan, Italy) after sub-boiling distillation performed with a sub-boiler SAVILLEX DST 1000 (Savillex Corp. USA) apparatus. All the solutions were gravimetrically prepared and all the samples were accurately weighted by using a Mettler AE200 analytical balance (Mettler Toledo AG, Greifensee, Switzerland) with ± 0.0001 g sensitivity.

All PFA bottles, tubes and vessels were cleaned with a solution of aqua regia (3:1, HCl and HNO_3), washed with heated HNO_3 10M and rinsed with high-purity water before use.

NIST SRM 987 $SrCO_3$, certified for its Sr isotope composition with a $^{87}Sr/^{86}Sr$ certified value of 0.71034±0.00026 [22] and a "generally accepted" one of 0.71026±0.00002 (the uncertainty is expressed as twice the standard deviation, 2s) [23], has been used for bracketing procedure as well as for the evaluation of the accuracy and precision of the obtained values. The standard stock solution of 200 μg kg^{-1} $SrCO_3$ was gravimetrically prepared using the NIST SRM 987. All working solutions were stored in PFA vessels (Nalgene).

NH_4NO_3 1M, Suprapur® (Merck, Milan, Italy), was used for the bio-available fraction extraction in soil.

The Eichrom Sr resin SR-B100-S (50-100 μm) was used for Sr/Rb matrix separation. In particular, 10 g of resin were conditioned in a 100 mL PFA bottle with approximately

50 mL of HNO_3 1% w/w. After overnight soaking, the supernatant was removed and the bottle was refilled with fresh HNO_3 1% w/w until the final content of the solution was at least 100 mL. The suspension ready for the use was stored at room temperature. Before each analysis, the bottle was shaken with an automatic end-over-end agitator (Rotator SB3, Stuart) for at least 30 min. Finally, 2 mL and 1 mL of the resin, for soils and vine branches/juices, respectively, were loaded in homemade SPE columns, namely polypropylene (Alltech, Milan, Italy) SPE extract clean TM reservoirs equipped with 20 µm polyethene frits (Alltech, Milan, Italy), by using a positive displacement micropipette (Gilson M1000) equipped with polyethylene piston and tips. The used resin was not recycled. Final Sr fraction solutions were collected in PFA tubes and analyzed in few days.

4.3.5. Analytical instrumentation

Strontium isotope ratio measurements were accomplished with an MC-ICP-MS spectrometer (Neptune, ThermoFinnigan, Bremen, Germany). The instrument consists of a double focusing, multicollector mass spectrometer with a forward Nier-Johnson geometry. The instrument is equipped with nine Faraday collectors, eight movable and a central fixed one. Data acquisition, performed in low resolution mode, was simultaneous for all the measured ion masses, m/z: 82 (L4), 83 (L3), 84 (L2), 85 (L1) 86 (C), 87 (H1), 88 (H2). The instrumental parameters are summarized in Table 4.6.
For each session of measurements, a gain calibration was performed for the multicollector system. Ion-lens setting was daily tuned to obtain the better compromise among the maximum sensitivity, instrumental stability and optimal flat-top peak shape.
The method for the analytical determination of $^{87}Sr/^{86}Sr$, such as sequence characteristics, calculation procedure and mathematical corrections, is described elsewhere [21].

Table 4.6. MC-ICP-MS operating parameters used for the 87Sr/86Sr measurements in samples.

Parameter	Value/Type
Rf Power	1245 W
Auxiliary gas flow rate	1.20 L min^{-1}
Cooling gas flow rate	16 L min^{-1}
Sample/Skimmer cone	Ni
Spray chamber	Cyclonic + Scott type
Nebulizer	PFA micro Flow Self aspirating
Number of block	1
Number of cycles	100
Integration time	8.839 s
Measure time for each sample	14 min
Uptake time	300 s
Wash time	100 s
Idle time	10 s
Mass analyzer pressure	< 10^{-8} mbar
Background/baseline determination	HNO_3 (4% w/w)
Control cup for peak centering	C

The digestion of grape juices and vine branches samples was accomplished by a MARSX microwave oven (CEM Corp., Milan, Italy) equipped with a 12 position carousel and 6 Teflon® XP-1500 Plus high pressure vessels, provided by the same company. Microwave apparatus is also equipped with a temperature probe (RTP-300 Plus) and a pressure sensor (ESP-1500 Plus) for pressure and temperature measurement on a reference vessel.

Leaching of soil with HNO_3 was done by means of a MW assisted procedure using the same instrument equipped with a 40 position carousel and 8 Xpress PFA vessels, provided by CEM. In this case, a contactless infrared vessel temperature control (DuoTemp, CEM Corp.) was activated for the temperature control inside all the vessels.

4.4. Results and discussion

4.4.1. $^{87}Sr/^{86}Sr$ for the *Lambrusco PDO* wine case of study

As mentioned in Section 4.2.1, only the upper (**a**) and lower (**e**) fractions for the in-plain soil samples (producers A, B and D) of the three sampling periods were analyzed in order to obtain the $^{87}Sr/^{86}Sr$ values relative to the extracted bio-available fraction. On the other hand, owing to the complexity which characterizes the hill site, all the depths of producer C samples were considered for the first sampling period, whilst only the upper and the lower ones were analyzed for the following samplings.

In Figure 4.4, the $^{87}Sr/^{86}Sr$ isotope ratios relative to the soil fractions extracted with NH_4NO_3 are reported, in order to evaluate the presence of peculiarities and/or trends in the data. An uncertainty equal to ± 0.00003 is associated to each value, calculated as twice the standard deviation of a replicated soil sample.

At first, it is evident a discrimination of the samples on the basis of their Sr-I.R. values. In particular, a first group is composed by in-plain samples (A, B, D) with values in the range of 0.70866 ÷ 0.70948; the other one is made of hill samples (C) which Sr-I.R. values from 0.71030 to 0.71146. This distinction is related to the different geology of the soils coming from the two areas and, according to the properties of the strontium isotope ratio, it is possible to state that hill soils, which present higher values, were formed from older rocks.

Moreover, it is possible to highlight a greater variability of the hill samples with respect to the in-plain ones, as regards both the sampling points and depths. In particular, the main differences are found for hole 4, which presents higher values than the others, and along the vertical profile of hole 5, with an increase in the isotope ratio from depth **a** to **e**. This last trend is quite peculiar and some researchers have described these isotope variations as function of the sampling depth, explaining the experimental evidences as a consequence of the different origin, and thus geology, of rock fragments which can produce stratification of soils.

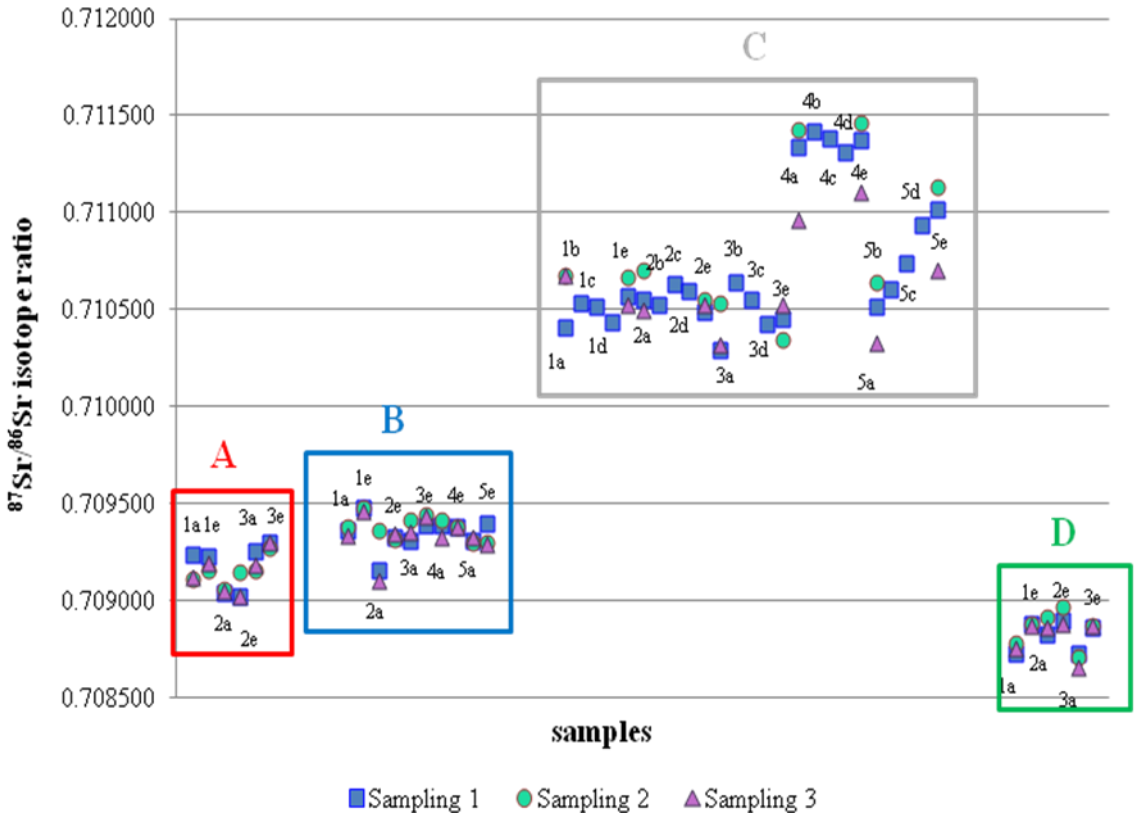

Figure 4.4. $^{87}Sr/^{86}Sr$ isotope ratios of bio-available fractions extracted with NH_4NO_3 from the soil samples of the four considered producers/fields (A, B, C and D). Symbols refer to the three sampling periods (1st sampling: □, 2nd sampling: ○ and 3rd sampling: △) and their widths are equal to the uncertainty associated to the measure (± 0.00003). Labels identify the sampling points in the field and the sampling depths, from **a** to **e**.

The Sr-I.R. values obtained for the bio-available fraction extracted from soil samples of in-plain producers result more homogeneous. A and B samples have quite similar isotope ratios, even if, considering the mean value for each producer, B field presents slightly higher values than the A one (A: 0.70916 vs. B: 0.70935). Samples of D producer are characterized by lower values (C: 0.70883).

Small differences are found among the sampling points of A producer, namely points 1 and 3 present higher values than point 2. Vertical variability seems negligible, since the isotope ratio of **a** and **e** fractions are nearly the same for all sampling points.

B producer's samples are characterized by limited variability both among the sampling points and depths, except for **a** fraction of point 2 (only first and third sampling), which presents lower values.

Variability along the depth profile is more emphasized for D samples, where **e** fractions are systematically higher than **a**. This leads to the inference that the lower soil layer (50 to 60 cm depth) could be made of older origin sediments.

Finally, seasonal variability should be taken into account. No common trends are found for samples of the different sampling periods (different symbols in Figure 4.4). Values obtained for almost all the in-plain samples in the three periods present differences within the measurement uncertainty. Exceptions are the 2**a** sample of producer B for the third sampling, 1**a** sample of producer A for the first sample and 2**e** sample of producer A for the second sampling, which all present higher values with respect to the relative samples of the other sampling periods. Here, differences are around the fourth decimal digit.

Quite complex situation occurs for hill samples, where differences among the different sampling periods can rise till 0.0004. However, the seasonal influence cannot be totally responsible for this variability, since no explicable behavior, common to the samples is found. The great heterogeneity of the site could be more probably the explanation of this phenomenon. Indeed, since the soil characteristics (geology and chemical composition) could change within a limited area, the not perfectly reproducible GPS position of the sampling points causes the occurrence of discrepancies in the samples collected at different times.

Hence, for the further comparisons, only the results relative to the first sampling period were considered as representative of the soil fraction extracted with NH_4NO_3.

These values were then compared with those obtained from the soil extraction with HNO_3 (Figure 4.5), obtained in a previous work [21]. The differences in the values obtained with the two extraction methods are here highlighted. If, on one hand, the values' dispersion for producers B and D is more or less the same and the Sr-I.R. is rather consistent, otherwise is for producers A and C. Indeed, the variability of the strontium isotope ratios considerably increases when considering the values obtained

with HNO_3 with respect to those relative to NH_4NO_3 extraction. In particular, as regards site A, a great variability is evident along the depth profile, since **a** and **e** fractions obtained with HNO_3 have quite different Sr-I.R. values. The NH_4NO_3 extraction flattens these differences.

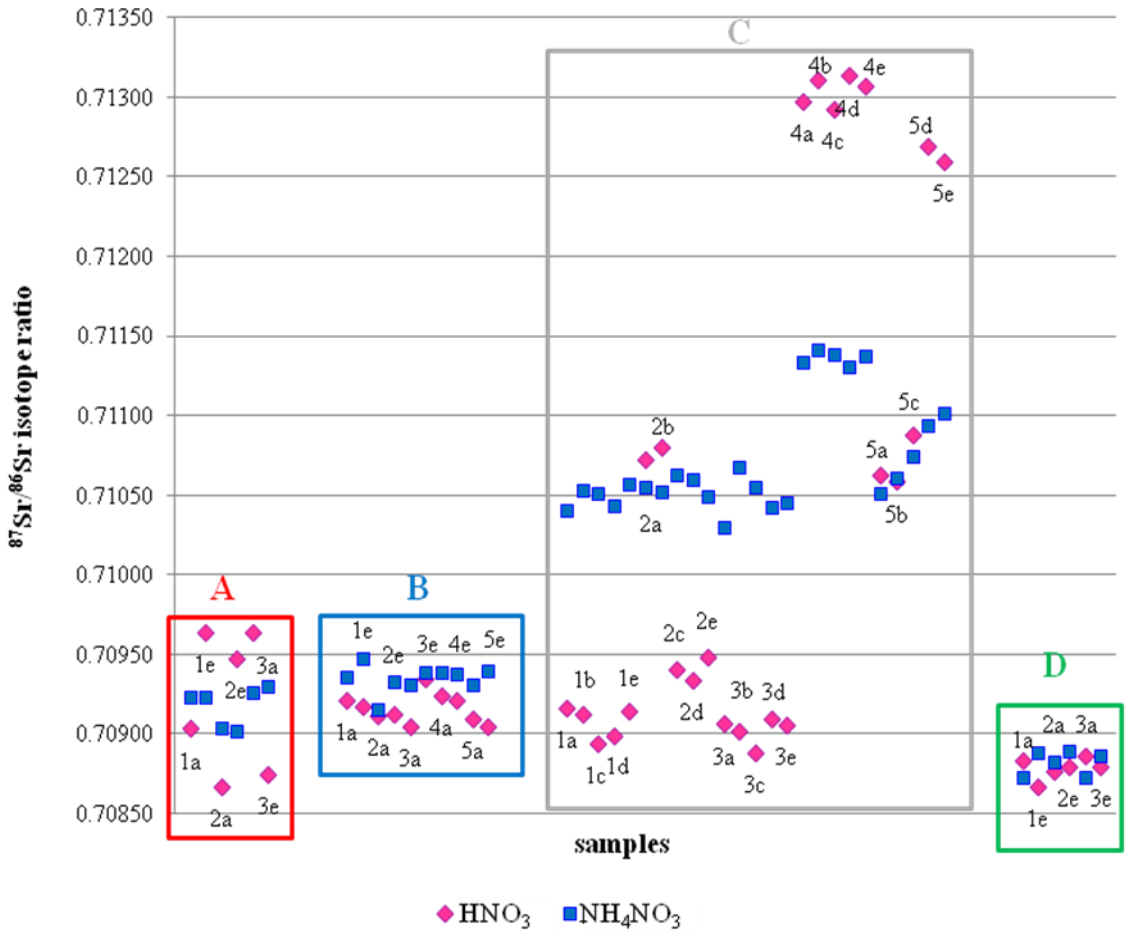

Figure 4.5. $^{87}Sr/^{86}Sr$ isotope ratios relative to the bio-available fraction extracted with NH_4NO_3 (□) and to the fraction extracted with HNO_3 (◊) from the soil samples of the four considered producers/fields (A, B, C and D). Only the first sampling period was considered. Labels identify the sampling points in the field and the sampling depths, from **a** to **e**. Symbol widths are equal to the uncertainty associated to the measure (± 0.00003).

Similarly, also the variability of producer C samples is large when considering the values obtained by HNO_3 extraction, whilst it is reduced, still remaining of a certain

entity, for the NH_4NO_3 extracts. In particular, the differences in the depth profile of point 2 are removed.

This situation can be probably explained as a consequence of the complex geology that characterizes some of the investigated areas, as described in Chapter 3. HNO_3 is classified as strong extractant, whilst NH_4NO_3 as mild one, meaning that the former is able to solubilize more chemical structures and thus different forms in which the strontium is present in the soil.

It is thus clear that the $^{87}Sr/^{86}Sr$ values of soils are deeply influenced by the geology of the area, the chemical form of the Sr in the soil and thus its mobility and bioavailability toward the plant roots.

Besides, since the main goal of this study is to find, or eventually verify, which strontium fraction better mimic the one present in the food, all the soil values were compared with those of the juices obtained from the grapes cultivated in proximity of each sampled point (Figure 4.6).

From the figure, it emerges that the $^{87}Sr/^{86}Sr$ values of the juices have different range of variability depending on their provenance and they are closer to Sr-I.R. obtained for the soil fraction extracted with NH_4NO_3 (bio-available fraction). This behavior is foreseeable, given that the NH_4NO_3 extraction is the generally used procedure for the bio-available fraction determination in soils. Therefore, the following considerations will only take into account these results.

Values obtained for juices belonging to B and D fields perfectly match with those monitored in the respective soil samples; in particular better correspondence is found with the lower depth (**a**). Differences are around the fifth decimal digit, thus not statistically significant since comparable to the uncertainty associated to the measurement. As far as the hill farm (C) and producer A are concerned, the variation between the Sr isotope composition of soil extracts (considering **a** depth) and that of grape juices is around the fourth decimal digit. In detail, $^{87}Sr/^{86}Sr$ of producer C juices ranges between 0.70978 and 0.71045 and the worst soil-juice correspondence is found for site 4 (difference is 0.00088). As concerns producer A, Sr-I.R. values vary from 0.70886 to 0.70898 and the maximum discrepancy (0.00028) is relative to site 1.

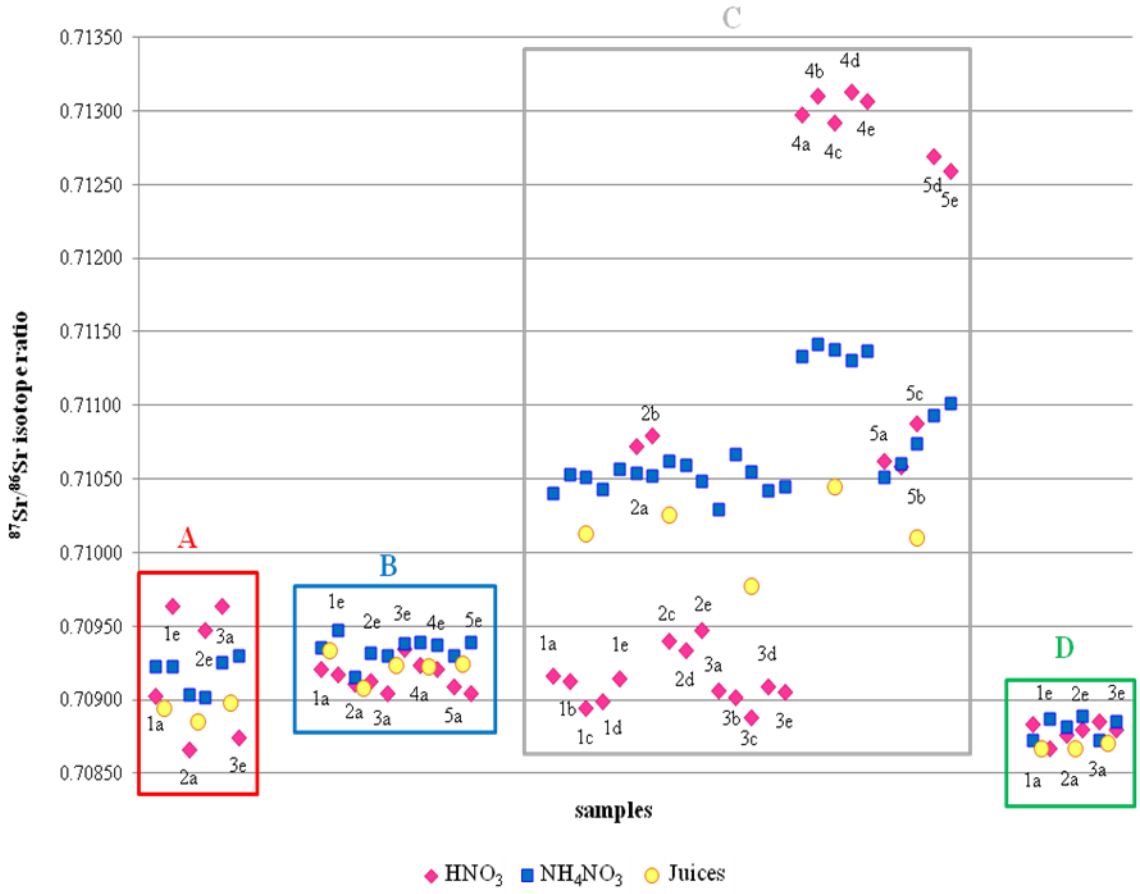

Figure 4.6. $^{87}Sr/^{86}Sr$ isotope ratios relative to soil samples and grape juices (○) of the four considered producers/fields (A, B, C and D). As regards soils, values refers to the bio-available fraction extracted with NH_4NO_3 (□) and to the fraction extracted with HNO_3 (◊). Only the first sampling period was considered. Labels identify the sampling points in the field and the sampling depths, from a to e. Symbol widths are equal to the uncertainty associated to the measure (± 0.00003).

On the basis of the described results, a good correlation exists between soils and juices for the investigated in-plain area. As concerns hill matrices, the situation is certainly more complex, since the discrepancies are rather higher than the precision of the measurement. Even if traceability models based on $^{87}Sr/^{86}Sr$ values with soil-food differences on the fourth decimal digit could be considered optimal [24–26], other aspects, which influence the correlation degree, could be highlighted.

Indeed, the differences found between Sr-I.R. of soils and of juices for hill samples are too big to be only due to procedure uncertainties or Sr fractionation in the soil (fractionation processes during the plant uptake are considered negligible). As a consequence, when the geology, structure and composition of soil is complex, the bio-available fraction extracted with NH_4NO_3 is not likely capable to perfectly reproduce the plant uptake.

Finally, in order to assess the trustworthiness of the previous inferences, the strontium isotope ratio was measured for the branches coming from the grapevines grown close to the sampling points. Vine branches were picked up during the first and the second sampling periods. In this way, it is possible to obtain information about the plant uptake, using the grapevine as direct sampler.

From Figure 4.7, it is evident the excellent correlation between Sr-I.R. measured in branches and juices. In fact, the differences for producer A are significantly reduced, all passing from the fourth to the fifth decimal digit and remain on the fifth decimal digit for producers B and D. As far as producer C is concerned, the branches-juices discrepancy certainly improves with respect to soils-juices, even if a perfect correlation is found only for point 1 and for point 2, when considering the mean value of the branches collected in the two periods. The differences for the other sampling points remain over the measurement uncertainty, although limited to a maximum value of $4\ 10^{-4}$.

The branches values for the two sampling periods are the same (differences not statistically significant) for almost all the cases. Peculiar situation occurs for the branches of point 2 of the producer C, which present very distant values, whose average perfectly matches the juice value.

These results confirm that the Sr uptake from minerals is not uniform, but it depends on many parameters such as the solubility, water content, mineral type, temperature, etc., in the root space of the plant. On the basis of these considerations, it became clearer the better similarity of values between juices and vine branches with respect to soils, confirming the "sampling capacity" of the plants in traceability studies.

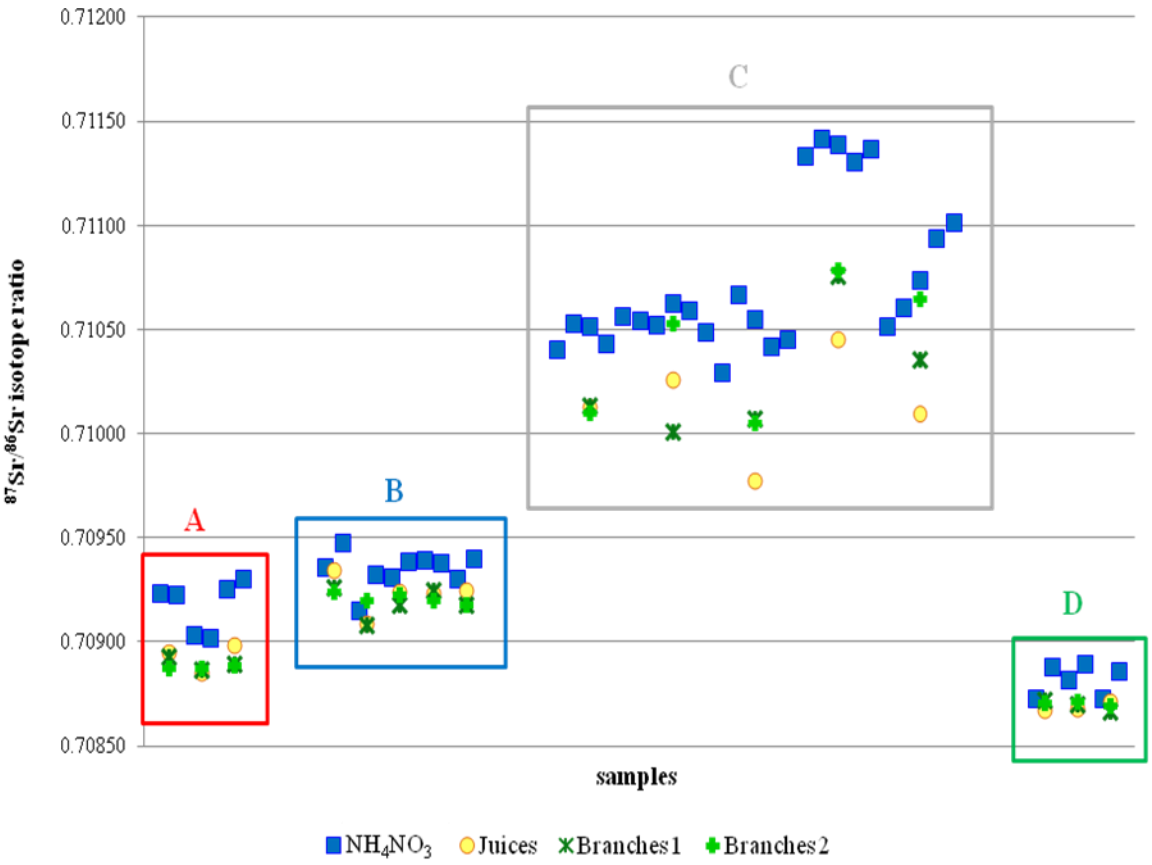

Figure 4.7. $^{87}Sr/^{86}Sr$ isotope ratios relative to soil samples, grape juices and vine branches of the four considered producers/fields (A, B, C and D). As regards soils, values refer to the bio-available fraction extracted with NH_4NO_3 for first sampling's samples. Symbol widths are equal to the uncertainty associated to the measure (± 0.00003).

4.4.2. $^{87}Sr/^{86}Sr$ for the *Chianti Classico DOCG* wine case of study

As described in Section 4.2.2, two fields (indicated as 1I and 2I) were considered for the *Chianti Classico* producer, located in Tuscany. Soil samples (divided in **up** and **down** fractions) and vine branches were collected from a total number of seven sampling points, whilst two pre-wine samples, referring to the two fields, were gathered from the producer some months after the vintage.

Therefore, Sr-I.R. values were determined on the solutions obtained by the NH_4NO_3 extraction, which could represent the bio-available element fractions, as well as on

those obtained by the complete digestion of vine branches. In this manner, it was possible to investigate the $^{87}Sr/^{86}Sr$ isotope ratio behavior in the grapevine system, taking into account the uptake ability of the plant.

Moreover, the indicator was measured on the pre-wine samples, in order to monitor the whole process.

All the measured $^{87}Sr/^{86}Sr$ values are reported in Figure 4.8.

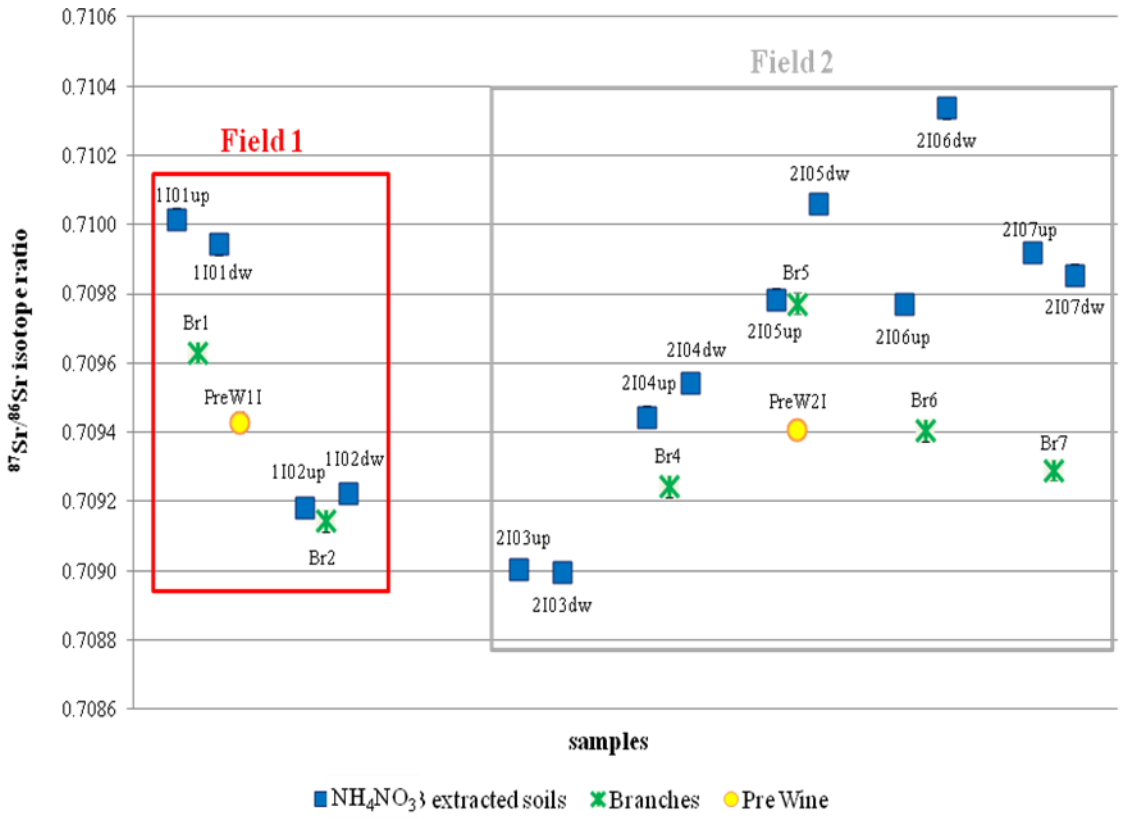

Figure 4.8. $^{87}Sr/^{86}Sr$ isotope ratios relative to soil samples extracted with NH_4NO_3, vine branches and pre-wines (PreW) of the two fields of the Chianti Classico producer. Symbol widths are equal to the uncertainty associated to the measure (± 0.00003).

Looking at the soil values, it is possible to highlight a great variability among the sampling points within each field of sampling (around the fourth decimal digit of the Sr-I.R. values). The obtained values are in accordance with the nature of the soil parent materials of the investigated area. Briefly, the *Chianti* production region can be

considered geologically heterogeneous. In fact, there are two main soil types: i) weathered sandstone (composed mainly by quartz and/or feldspar) known as *Alberese* and a bluish-gray chalky marlstone (a form of limestone composed by mineral calcite) known as *Galestro*, which both should present strontium isotopic variability in agreement with the current data [27, 28].

Notwithstanding, the differences between the **up** and **down** (dw) fractions for each sampling point cannot be considered statistically significant since they are comparable with the uncertainty associated to the measurements. Exceptions can be found for point 5 and, in particular, for point 6 (differences between **up** and **dw** samples of 0.0002 and 0.0004, respectively).

Finally, the following strontium isotopic variability ranges (average ± standard deviation) can be defined for both the sampled fields: 0.7096 ± 0.0004 for field 1 (sampling points 1I01 and 1I02), and 0.7097 ± 0.0004 for field 2 (sampling points from 2I03 to 2I07).

Branches collected from the vine surrounding each sampling point present values, which are statistically different from those of the soil in most of the cases. This could be probably due to the complexity of the geology of the soils as well as of the factors influencing solubility and form of available metal, which leads to a not precise determination of the bio-available fraction of soils with the used chemical medium.

Nevertheless, considering the variability ranges for the vine branches of the two fields are 0.7094 ± 0.0003 and 0.7094 ± 0.0002, respectively, they match with their respective fields of provenance.

As far as the pre-wines are concerned, the isotopic values obtained for PreW1I and PreW2I are 0.70943 and 0.70941, respectively. Both values perfectly agree with the respective isotopic variability ranges found for soils and branches. However, considering the standard deviations, branches values show a better discriminating ability than the soil, being characterized by a lower variability. These evidences support the inference that the plant could play a relevant role, mediating the element uptake process from soil. The evaluation of the bio-available fraction, performed with extraction agents, could cause deviations from the real uptake and thus among the values obtained

for soil, plant and final product. The effects and the entity of these discrepancies are related to the peculiar structure and composition of the soil.
Unfortunately, the data relative to this particular case could be considered preliminary and suggest to perform a more detailed investigation, mainly based on the plant-final product system.

4.4.3. $^{87}Sr/^{86}Sr$ for the *Barolo DOCG* wine case of study

As regard the *Barolo* producing area in Piedmont, two producers were taken into account. In this case a more focused and punctual study is needed, since the perceived quality of the wine is related to the producer's brand, limited territory and "savoir faire".
For the first producer, only one field was considered with a total number of eleven sampling points. Four pre-wines were gathered as the production of the whole field.
The $^{87}Sr/^{86}Sr$ values measured on the bio-available fractions, extracted with NH_4NO_3 from soil samples, and on the solution obtained by digestion of pre-wine samples are reported in Figure 4.9.
It is possible to notice a great homogeneity in the soil values of the field, with respect to the previous Tuscany case. Also the depth variability is lower than the measurement uncertainty, except for points 6 and 9. The soil variability range for the first *Barolo* producer is 0.7092 ± 0.0001 and results to be in accordance with the soil features of the *Barolo* producing region [28]. As a matter of fact, that area is characterized by the presence of silty-clay soils, rich in calcium carbonate (*Marl*) [29].
The values measured for the relative pre-wines are also rather similar, presenting a range of 0.70914 ± 0.00008, which perfectly lies within the respective soil one.

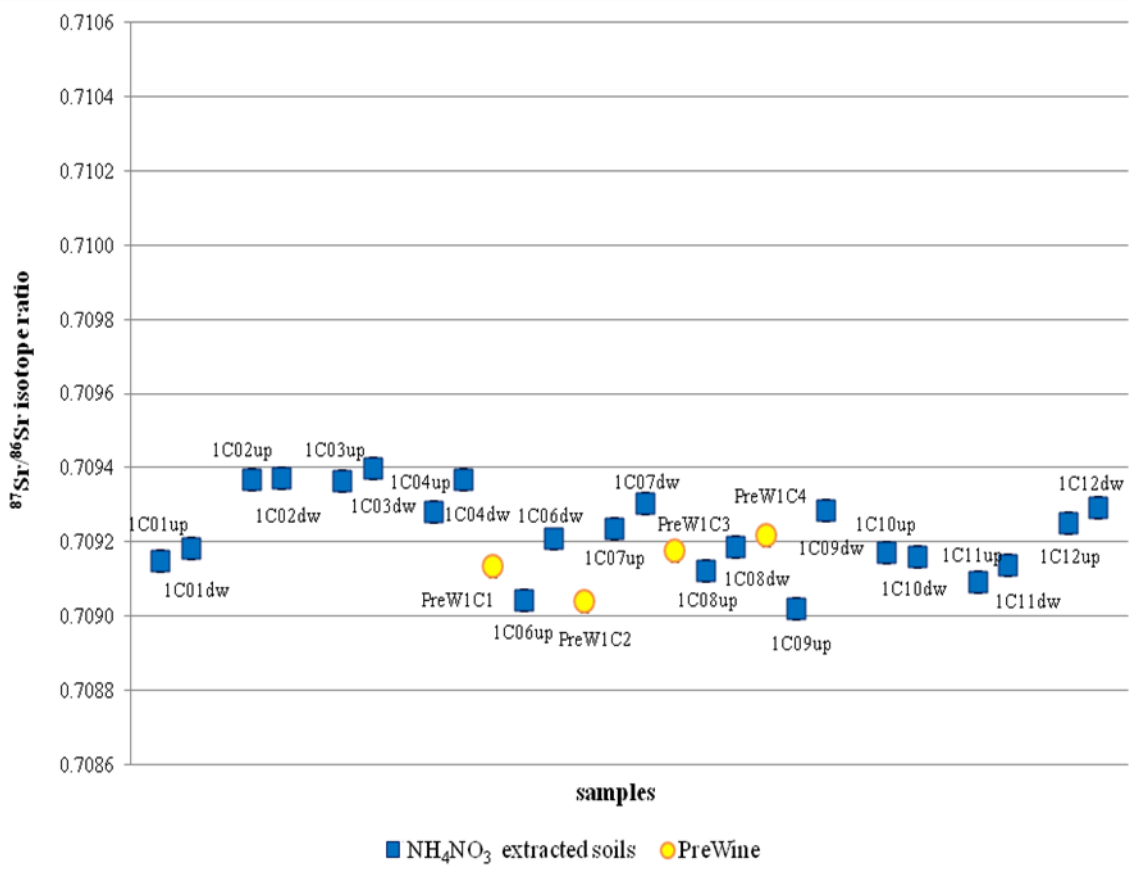

Figure 4.9. $^{87}Sr/^{86}Sr$ isotope ratios relative to soil samples extracted with NH_4NO_3 and pre-wines (PreW) of the first *Barolo* producer. Symbol widths are equal to the uncertainty associated to the measure (± 0.00003).

As far as the second *Barolo* producer is concerned, two fields were taken into account, giving a total number of fifteen sampling points. Six pre-wines were collected from the producer, with indications about the approximate location of the vines the grapes come from (Section 4.2.3). The results of the $^{87}Sr/^{86}Sr$ determination on these samples are reported in Figure 4.10. Similarly to the other producer, soils are quite homogeneous, with variability ranges for the two fields of 0.7091 ± 0.0002 and 0.70913 ± 0.00009, respectively. Hence, field 1 presents a slightly wider values distribution.

The Sr-I.R. measured for PreW1M (0.70930) lies within the soil range, but shifted to the higher values. This behavior could be probably explained by considering that the

final product is given by the harvest coming from the whole field, hence the pre-wine value is a weighted average.

As regards the second fields, the five pre-wines produced with grapes coming from different areas present almost identical values, meaning that the variation is within the measurement uncertainty. The pre-wine variability range is 0.70906 ± 0.00002 and it perfectly matches the range of the soils.

The low variability of the values of these two *Barolo* producers is important in order to obtain discrimination of their products. For this reason, the possibility to directly measure the isotope ratio of the grapevines growing in the area could allow to further reduce the values dispersion, leading to more detailed traceability models.

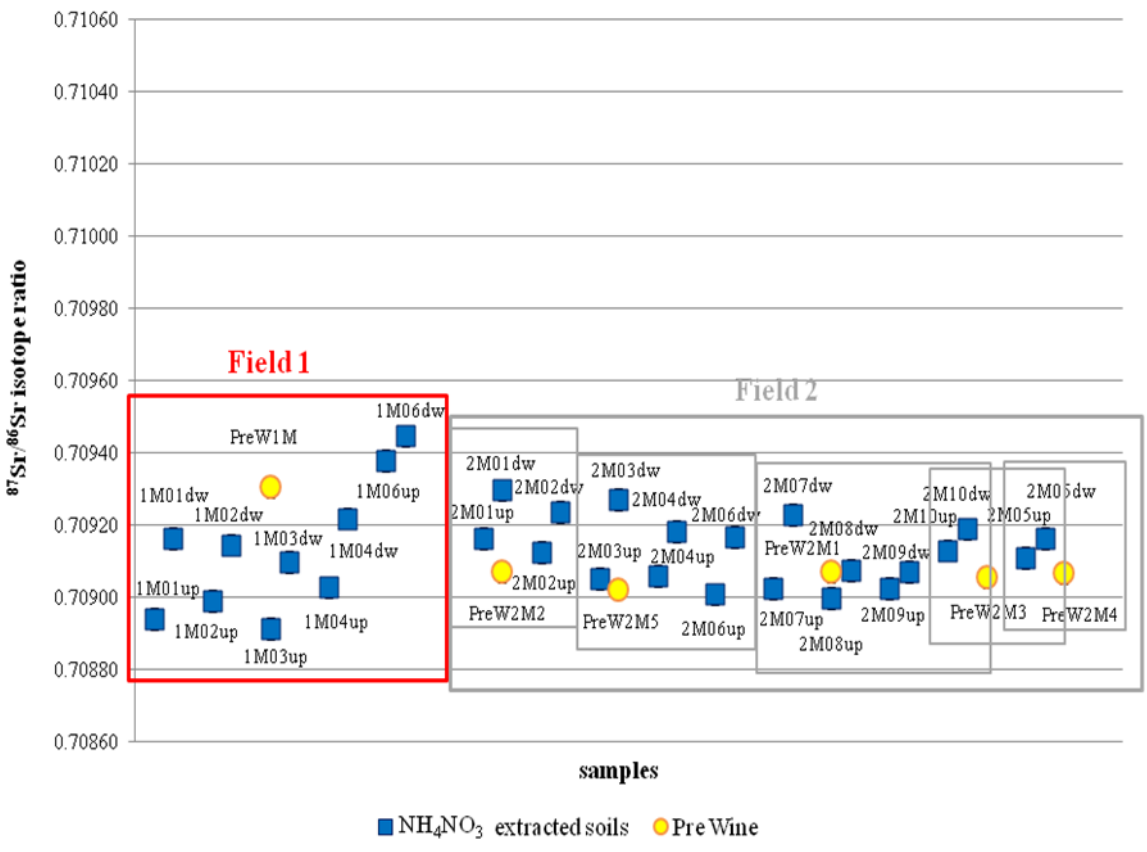

Figure 4.10. $^{87}Sr/^{86}Sr$ isotope ratios relative to soil samples extracted with NH_4NO_3 and pre-wines (PreW) of the second *Barolo* producer. Symbol widths are equal to the uncertainty associated to the measure (± 0.00003). Squares indicate which soil samples the pre-wine refers.

4.5. Conclusions

The first example presented in this chapter is part of a long term research project focused on the development of authenticity and geographical traceability models of oenological products, in particular *Lambrusco PDO* wines. The second and the third cases refer to a parallel study on more restricted areas, such as a *Chianti Classico DOCG* and two *Barolo DOCG* producers.

Notwithstanding the different adopted strategies and abundance of information, the common aim is to investigate the indicator (in this case $^{87}Sr/^{86}Sr$ isotope ratio) variability in the area and to monitor its behavior within the soil-final product system.

In the *Lambrusco DOP* case, in particular, the $^{87}Sr/^{86}Sr$ was measured on the bio-available fraction, extracted with NH_4NO_3 from soils, in order to obtain information about the inter- and intra-site variability and also along the vertical profile. Then, the indicator was determined also on the grape juices, to verify the correspondence between the values measured in the soils and in the juices, and on vine branches, to evaluate the ability of the chemical extractant in mimicking the plant uptake.

The results of the soils extracts were initially compared with previous ones obtained for the same soils extracted with HNO_3, in order to assess the differences occurring with different extraction media. Discrepancies are present in some cases, in particular for the hill producer, probably due to the complex structure and composition of soils, which present strontium in several forms. The extraction media are differently able to extract strontium on the basis of its chemical form, leading to different isotopic signatures.

Sr-I.R. values measured in the juices are closer to those of the mobile fraction extracted with NH_4NO_3 than with HNO_3. Nevertheless, in the case of hill area, the extracted bio-available fraction could not sufficiently reproduce the real uptake of the plant and hence the Sr-I.R. in the food. Indeed, some differences found between the soil and grape juice values are too high to be considered as measurement errors or fractionation processes (this phenomenon is negligible for heavy isotopes). Hence, the complexity of the soil could deeply influence the plant uptake making difficult to obtain a precise reproduction with chemical agents.

In this case, a great support is found by directly determining the $^{87}Sr/^{86}Sr$ values in the plants from which the grapes come. In fact, it is more plausible to think that there is a higher correspondence between the monitored Sr-I.R. of plant and food, rather than of soil and food. The obtained results prove this hypothesis, showing an optimal branches-juices match.

The other two examples confirm the same considerations on more restricted sites located in different Italian areas.

In summary, the $^{87}Sr/^{86}Sr$ isotope ratio is certainly a powerful geographical traceability marker, owing to its peculiarities which allow discriminating, both horizontally and vertically, soils samples with different origins and characteristics.

However, the great variability of the indicator could be also considered as a drawback, since it contributes to data dispersion, leading to a more difficult distinction of samples coming from different geographical areas.

The possibility to use the plant as sampling device could reduce the dispersion of the data, allowing a better discrimination of the samples. Further, by considering the branches values, it could be possible to overcome problems associated with soil sampling and its structure and composition, as well as those related to the evaluation of the bio-available fraction of soil, namely the plant uptake.

The presented approach could be considered very promising in geographical traceability studies.

4.6. References

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CHAPTER 5

Variation of the strontium isotope ratio during the winemaking process

5.1. Introduction

As discussed in the previous chapters, the development of geographical traceability models, able to find out a relationship among the $^{87}Sr/^{86}Sr$ values monitored in the territory of provenance and in the food product, requires a deep knowledge of the whole investigated system and its peculiarities.

Besides the phenomena which could influence the strontium isotope ratio transferring from the soil to the grapes (Chapter 4), also the following procedures, leading from the raw materials to the final products, should be analyzed.

In fact, the winemaking process comprises several steps which may introduce changes in the element pattern, due to both natural and anthropogenic/extraneous sources.

Several studies have been conducted on the variation of some element concentrations during the cellar practices [1–10]. In particular, storage tanks, pipes and other wine cellar equipments (brass and stainless steel) increase the content of Cd, Cr and Pb [4] or Fe, Al and Cr [7]. Significant contributions to Al [2], Rare Earth Elements (REEs) [3] [8] and exchangeable cations, such as Mg, Ca and Na [6] are found, due to the additives used for purification or deacidification which could be sources of metals.

Variations in the element content occur also as a consequence of the fermentation process [1, 6].

Since the winemaking process produces changes in the elemental concentrations, an accurate study to determine whether the strontium isotope ratio is also influenced is necessary in order to finally achieve a geographical traceability of wine on the basis of this indicator.

If no sources of "external" strontium are added, an increase or decrease in the strontium concentration should not directly affect the isotope ratio. In any case, it is worth investigating whether and which strontium contaminations occur during the vinification.

An increase of strontium is found in the first steps of the winemaking process, probably released by seeds and skins, whilst a slight decrease during the aging period is a consequence of strontium precipitation with colloidal particles [11]. Therefore, these two phenomena are natural, not being due to external sources, and should not affect the

$^{87}Sr/^{86}Sr$ isotope ratio. On the contrary, the addition of bentonites, used for wine clarification, or calcium carbonate, to deacidify, could be the main extraneous sources of strontium [12].

The only attempt to investigate the influence of the winemaking process on the strontium isotope ratio [11] reveals no differences in the values measured in the different phases. Nevertheless, it is worth noting that in the previous investigation was used an ICP-qMS for the evaluation of the strontium isotope ratio. Unfortunately, this instrument is characterized by a precision (in terms of relative standard deviation, RSD%) reaching 0.01%, which is not enough to eventually discriminate differences among samples. Consequently considering the precisions obtained with HR-MC-ICP-MS, around 0.001%, an updated study on the behavior of the $^{87}Sr/^{86}Sr$ indicator during the vinification is needed.

Moreover, since the production procedures can be different as function of the type of wine, wineries and countries, tailored investigations are suggested, in order to evaluate the indicator behavior.

Aim of the study presented in this chapter is to monitor the $^{87}Sr/^{86}Sr$ isotope ratio trend within the winemaking chain and assess whether and how the production process can cause variations in the measured values. Samples for each step of the process (grape juice, intermediate products, wine) were tracked for seven *Lambrusco* winemaking chains, coming from four Modena district wineries, and nine *TrentoDoc* winemaking chains, coming from five Trento district wineries.

These productions are typical and regulated by stringent production laws [13–16], which allow the grape cultivation and each step of the production procedure only within limited areas.

5.2. Sampling and samples

5.2.1. *Lambrusco* winemaking chains

As regards the *Lambrusco PDO* wine, seven productions of four wineries were monitored in the period from September 2011 to April 2012 (or to the wine bottling date). A representative sampling of i) grape juices, obtained from Lambrusco Sorbara or Grasparossa grapes, ii) intermediate products of each production step and iii) final commercial *Lambrusco* wines, where available, was performed. For each batch, a sample aliquot was systematically collected after every variation occurred during the process, i.e. filtration, use of additives, mixing of the content of different silos, etc. The additives, used in the Lambrusco productions to make the wine sparkling, namely rectified concentrated must (MCR), red concentrate, etc., were also sampled, where available. All the collected samples were stored at 0 °C till the moment of the analysis.
Since each production has its peculiarities, steps and different amount of information, the winemaking chains of the four wineries and all the collected samples are below described.

PB winery

For this producer, only one batch was monitored and the samples with relative information are reported in Table 5.1.

Table 5.1. Collected samples for the PB winemaking chain

Sample code	Information
JAG_PB1a	must coming from the first silo after filtration
JAG_PB1b	must coming from the second silo after filtration
JAG_PB2ab	mixing of samples 1a and 1b at the end of the fermentation process
JAG_PB3ab	fermented samples after SO_2 addition
JAG_PB4ab	commercial Lambrusco Sorbara wine

The starting must comes from the juice obtained by Lambrusco Sorbara grapes and is initially stored in two silos with 300 hL capacity. After the mechanical harvesting, crushing is directly performed on field, where the grapes are put into a destemming machine, conveying must and grape skins in the collecting tank. A small amount of SO_2 is added to delay fermentation processes. In the winery, the must is stored into two silos, added with enzymes and grape tannins to facilitate skins separation and clarification, and finally roughly filtered (JAG_PB1a and JAG_PB1b).
The content of the two silos is then mixed in a fermentation tank, 500 hL, and added with yeasts. After about one week, the fermentation process stops owing to the complete transformation of the sugar substrate (JAG_PB2ab). The fermented sample is added with SO_2 and transferred in an autoclave for aging process (JAG_3ab). Then, different products, i.e. MCR or red concentrate, can be added to allow the wine to be sparkling. Finally the wine is bottled (JAG_PB4ab).

MO winery

This production makes use of Lambrusco Grasparossa grapes. The collected grapes are taken to the winery to be put in a mechanical crusher/destemmer. The grape must is stored and fermented with its skins within a concrete tank. Later, skins are removed and yeasts are added to start the fermentation. After about one month, the fermentation process ends and clarifying substances are added to the wine, i.e. bentonites, polyvinylpolypyrrolidone (pvpp) and gelatin. The wine ages for a period and then is bottled. The samples collected for this producer are reported in Table 5.2.

Table 5.2. Collected samples for the MO winemaking chain

Sample code	Information
JAG_MO1	grape must
JAG_MO2	must during the fermentation process
JAG_MO3	fermented must, added with bentonites, pvpp, gelatin
JAG_MO4	commercial Lambrusco Grasparossa wine

CE and CR winemaking chains

For this winery, three batches of two winemaking chains (two CE and one CR batches) were monitored (Table 5.3). The mainly used grape varietal is Lambrusco Grasparossa. In particular, the final CR product is made by 100% Lambrusco Grasparossa grapes, whilst the CE wine can contain up to 15% of other Lambrusco varietals.

After the harvest, the grapes are crushed and the must is transferred in a 1500 hL wine tank. The must is filtered with perlites, and then the fermentation starts by using selected yeasts. At the end of the fermentation process, must is centrifuged and added with rectified concentrated must. The product is stabilized at low temperature, subjected to tangential filtration and finally bottled.

Table 5.3. Collected samples for the CE and CR winemaking chains

Sample code	Information
first CE batch	
JAG_CE1a	grape must
JAG_CE2a	must after perlite filtration
JAG_CE3a	fermented must, after centrifugation
second CE batch	
JAG_CE1b	grape must
JAG_CE2b	must after perlite filtration
JAG_CE3b	fermented must, after centrifugation
CR batch	
JAG_CR1	grape must
JAG_CR2	must after perlite filtration
JAG_CR3	must waiting for the fermentation process to start
JAG_CR4	fermented must after low temperature stabilization
JAG_CR5	product after tangential filtration
JAG_CR6	Lambrusco Grasparossa wine

As regards the two CE batches, some problems occur during the winemaking process, leading to products with not appropriate characteristics to obtain a Lambrusco wine. For this reason, no commercial Lambrusco wine is available, being these products used to produce low quality wines.

CM winery

In this winery, two winemaking chains were monitored. The first one comprises two starting silos of Lambrusco Sorbara grape must, whilst the second one is made by five starting silos of Lambrusco Grasparossa grape must, mixed together at different steps of the production procedure (Table 5.4).

The grapes are crushed in the winery and stored in silos, with addition of SO_2, pectolytic enzymes, gelatins and N_2 to facilitate the separation of skins and other waste material from the must. After, the must is transferred in a fermentation tank for about a week till the end of the fermentation process, when the must is centrifuged to remove yeasts and particulates. Then, the low temperature stabilization is performed, sweet must (PAG) is added and the product is filtered by perlite and then diatomaceous earth. Finally the product is bottled.

Table 5.4. Collected samples for the CM winemaking chains

Sample code	Information
first CM batch	
JAG_CM1a	first silo of Lambrusco Sorbara grape must, added with SO_2
JAG_CM1b	second silo of Lambrusco Sorbara grape must, added with SO_2
JAG_CM2ab	mixing of 1a and 1b samples, after flotation process and starting fermentation
JAG_CM3ab	must at the end of the fermentation process
JAG_CM4ab	sample after centrifugation
JAG_CM5ab	sample after low temperature stabilization, with addition of PAG_CM1
JAG_CM6ab	commercial Lambrusco Sorbara wine

Table 5.4. (continued) Collected samples for the CM winemaking chains

Sample code	Information
second CM batch	
JAG_CM1c	first silo of Lambrusco Grasparossa grape must, added with SO_2
JAG_CM1d	second silo of Lambrusco Grasparossa grape must, added with SO_2
JAG_CM1e	third silo of Lambrusco Grasparossa grape must, added with SO_2
JAG_CM1f	fourth silo of Lambrusco Grasparossa grape must, added with SO_2
JAG_CM1g	fifth silo of Lambrusco Grasparossa grape must, added with SO_2
JAG_CM2c	1c must, after flotation process and starting fermentation
JAG_CM2de	mixing of 1d and 1e musts, after flotation process and starting fermentation
JAG_CM2fg	mixing of 1f and 1g musts, after flotation process and starting fermentation
JAG_CM3c	fermented 2c must
JAG_CM3de	fermented 2de must
JAG_CM3fg	fermented 2fg must
JAG_CM4cde	mixing of 3c and 3de samples, after centrifugation
JAG_CM4fg	3fg sample, after centrifugation
JAG_CM5cdefg	mixing of 4cde and 4fg samples, after low temperature stabilization with addition of PAG_CM1
JAG_CM6cdefg	commercial Lambrusco Grasparossa wine
additives	
PAG_CM1	filtered must of Sorbara grapes
PAG_CM2	filtered must of Ancellotta grapes

5.2.2. *TrentoDoc* winemaking chains

As regard the *TrentoDoc* wine, nine winemaking chains of five producers were selected and examined. No final commercial wine is still available for these productions since the bottled wine should age for at least fifteen months before being sold.

For these winemaking chains, detailed information about the cellar practices is not available at the moment and only a brief description of each sample and the respective grape varieties are reported in the following tables.

Table 5.5. Collected samples for the first *TrentoDoc* producer

Sample code	Information	Grapes varieties
JAG_PI1	grape juice	Chardonnay
JAG_PI2	must with addition of SO_2	Chardonnay
JAG_PI3	filtered must	Chardonnay
JAG_PI4	vine before aging process	Chardonnay

Table 5.6. Collected samples for the second *TrentoDoc* producer

Sample code	Information	Grapes varieties
JAG_AN1	grape juice	Chardonnay
JAG_AN2	must with addition of SO_2	Chardonnay
JAG_AN3	filtered must	Chardonnay
JAG_AN4	wine before aging process	Chardonnay

Table 5.7. Collected samples for the third *TrentoDoc* producer

Sample code	Information	Grapes varieties
SG batch		
JAG_SG1	drained and pressed must	Chardonnay
JAG_SG2	clarified must	Chardonnay
JAG_SG3	wine before aging process	Chardonnay
SP batch		
JAG_SP1	drained and pressed must	Pinot Nero
JAG_SP2	clarified must	Pinot Nero
JAG_SP3	wine before aging process	Pinot Nero
SC batch		
JAG_SC1	drained must	Chardonnay
JAG_SC2	clarified must	Chardonnay
JAG_SC3	wine before aging process	Chardonnay

Table 5.8. Collected samples for the fourth *TrentoDoc* producer

Sample code	Information	Grapes varieties
MS batch		
JAG_MS1	grape juice	Chardonnay
JAG_MS2	clarified must	Chardonnay
JAG_MS3	wine before aging process	Chardonnay
MR batch		
JAG_MR1	must after settling	Pinot Nero
JAG_MR2	must with yeasts	Pinot Nero
JAG_MR3	wine before aging process	Pinot Nero

Table 5.9. Collected samples for the fifth *TrentoDoc* producer

Sample code	Information	Grapes varieties
FP batch		
JAG_FP1	grape juice	Pinot Nero
JAG_FP2	pressed must	Pinot Nero
JAG_FP3	wine before aging process	Pinot Nero
FC batch		
JAG_FC1	drained must	Chardonnay
JAG_FC2	pressed must	Chardonnay
JAG_FC3	wine before aging process	Chardonnay

All the collected samples were stored at 0 °C till the moment of the analysis.

5.3. Experimental

5.3.1. Digestion procedure of grape juices, musts and wines

The digestion of grape juices, musts and wines was performed on a maximum sample aliquot of 6 g, accurately weighted into a quartz vial and then added with 4 mL HNO_3.

This mixture is left to react for at least 30 minutes, before the microwave digestion in autoclave. 5 vials (40 mL), fitted with Teflon caps, were placed in the rack and the mineralization cycle is set as shown in Table 5.10. A control samples was repeated once a day, to monitor the procedure performances.

Table 5.10. Microwave program used for the digestion of organic matrices

	Max power (watt)	**Ramp (min)**	**Temperature (°C)**	**Time @ Temp (min)**
1 step	1200	5:00	100	00:00
2 step	1200	15:00	210	05:00

At the end of the digestion process, all the samples were transferred in PFA bottles and diluted with 65% HNO_3 in order to obtain solution with 8M HNO_3 content and a suitable Sr concentration for the further analysis.

5.3.2. Interference separation

The isobaric interference of ^{87}Rb is usually minimized by means of a SPE (Solid Phase Extraction) separation [17], since it could highly affect the $^{87}Sr/^{86}Sr$ determination. The reliability of the separation procedure is thus extremely important to achieve precise and accurate measurement of the isotope ratio.

The Sr/Rb separation for organic matrices (juices, musts, wines) was optimized by means of Design of Experiment (DoE) methods [18] and basically consists of the following steps: i) 1 mL of resin suspension is loaded into the SPE column; ii) the resin is washed with 2 mL of high-purity water and activated with 5.5 mL of 8M HNO_3; iii) 10 mL of sample in 8M HNO_3 are loaded; iv) the interferences are eluted with 3.5 mL of 8M HNO_3 and v) the recovery of Sr is accomplished by using 7.5 mL of high-purity water. At the end of the separation procedure, a proper aliquot of 65% HNO_3 was added to all the water eluted fractions to obtain 4% HNO_3 final solutions.

5.3.3. Reagents and materials

All the sample preparation procedures were carried out under horizontal laminar flow hood equipped with an HEPA filter, in order to prevent the occurrence of any ambient contamination. Solutions were prepared by using high-purity deionized water TYPE1 (physical and chemical parameters for TYPE1 water comply with ASTM TYPE I and ISO3696 GRADE I purity specifications) obtained from a Milli-Q system (Millipore, Bedford MD) with a resistivity better than 18 MΩcm. Ultrapure HNO_3 65% w/w was obtained from analytical grade nitric acid (Carlo Erba, Milan, Italy) after sub-boiling distillation performed with a sub-boiler SAVILLEX DST 1000 (Savillex Corp. USA) apparatus. All the solutions were gravimetrically prepared and all the samples were accurately weighted by using a Mettler AE200 analytical balance (Mettler Toledo AG, Greifensee, Switzerland) with ± 0.0001 g sensitivity.

All PFA bottles, tubes and vessels were cleaned with a solution of aqua regia (3:1, HCl and HNO_3), washed with heated HNO_3 10M and rinsed with high-purity water before use.

NIST SRM 987 $SrCO_3$, certified for its Sr isotope composition with a $^{87}Sr/^{86}Sr$ certified value of 0.71034±0.00026 [19] and a "generally accepted" one of 0.71026±0.00002 (the uncertainty is expressed as twice the standard deviation, 2s) [20] has been used for bracketing procedure as well as for the evaluation of the accuracy and precision of the obtained values. The standard stock solution of 200 μg kg^{-1} $SrCO_3$ was gravimetrically prepared using the NIST SRM 987. All working solutions were stored in PFA vessels (Nalgene).

The Eichrom Sr resin SR-B100-S (50-100 μm) was used for Sr/Rb matrix separation, as described in the previous chapter (Section 4.3.3).

5.3.4. Analytical instrumentation

Strontium isotope ratio measurements were accomplished with an MC-ICP/MS spectrometer (Neptune, ThermoFinnigan, Bremen, Germany), as described in the previous chapter (Section 4.3.5).

The digestion of grape juices, musts and wines was performed by means of UltraWAVE Benchtop Single Reaction Chamber Digestion System (Milestone Inc., CT, USA).
At the heart of the UltraWAVE system is a Teflon lined, 1L stainless steel reaction chamber, which is also the microwave cavity. This allows the design of the 1500 W microwave source to be perfectly matched to the cavity shape for optimum microwave distribution and fast, even heating. Samples are weighed into vials and placed in a rack, which is lowered into the reaction chamber. The chamber is sealed and pre-pressurized with inert gas (N_2), which acts as a "cover" over the samples, preventing cross contamination. Unlike closed vessel digestion, different sample matrices can be digested together, and the chamber is water cooled, which makes cool down time very fast. Moreover, every sample is under direct pressure and temperature control; no need to rely on indirect control such as infrared temperature sensors. This assures complete control of the digestion process in every sample.

5.4. Results and discussion

5.4.1. *Lambrusco* winemaking chains

$^{87}Sr/^{86}Sr$ isotope ratio was determined on the grape juices, intermediate products and commercial wine for all the investigated winemaking chains.
A measurement uncertainty of ± 0.00003 is associated to each value, calculated as twice the standard deviation of a replicated sample.
The data obtained for the first six batches were reported in Figure 5.1, whilst those of the last batch of CM winery are in Figure5.2. From these graphs, some general consideration can be done. Sr-I.R. values are in agreement with the range found in the Modena district pilot study (Section 4.4.1). Those related to Lambrusco Grasparossa variety (MO, CE, CR batches and the second CM batch) are on average higher than those of the Lambrusco Sorbara (PB and first CM batch). Indeed, the cultivation area of Lambrusco Grasparossa grapes is located in the southern part of the Modena district, which comprises hill and in-plain territory, whilst Sorbara variety is mainly cultivated in

in-plain regions. Generally a rather constant Sr-I.R. value is found, but some exceptions and peculiarities in the winemaking chains require a more detailed discussion.

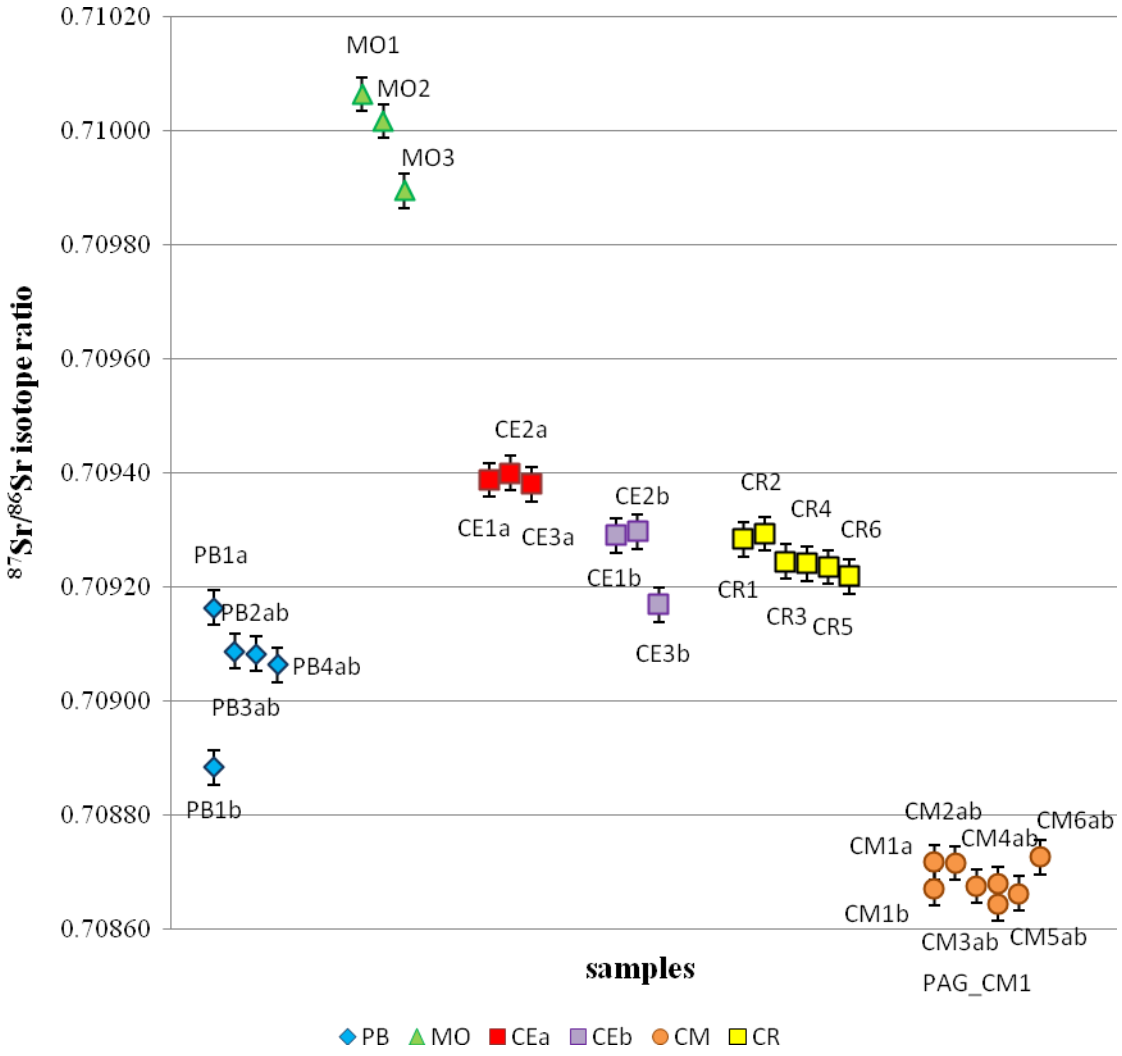

Figure 5.1. Graphical representation of the $^{87}Sr/^{86}Sr$ isotope ratios, measured for the first six wine batches of *Lambrusco* wine, namely PB, MO, CE and CR productions and the first batch of CM winery. The error bars refer to the uncertainty associated to the measurement (± 0.00003).

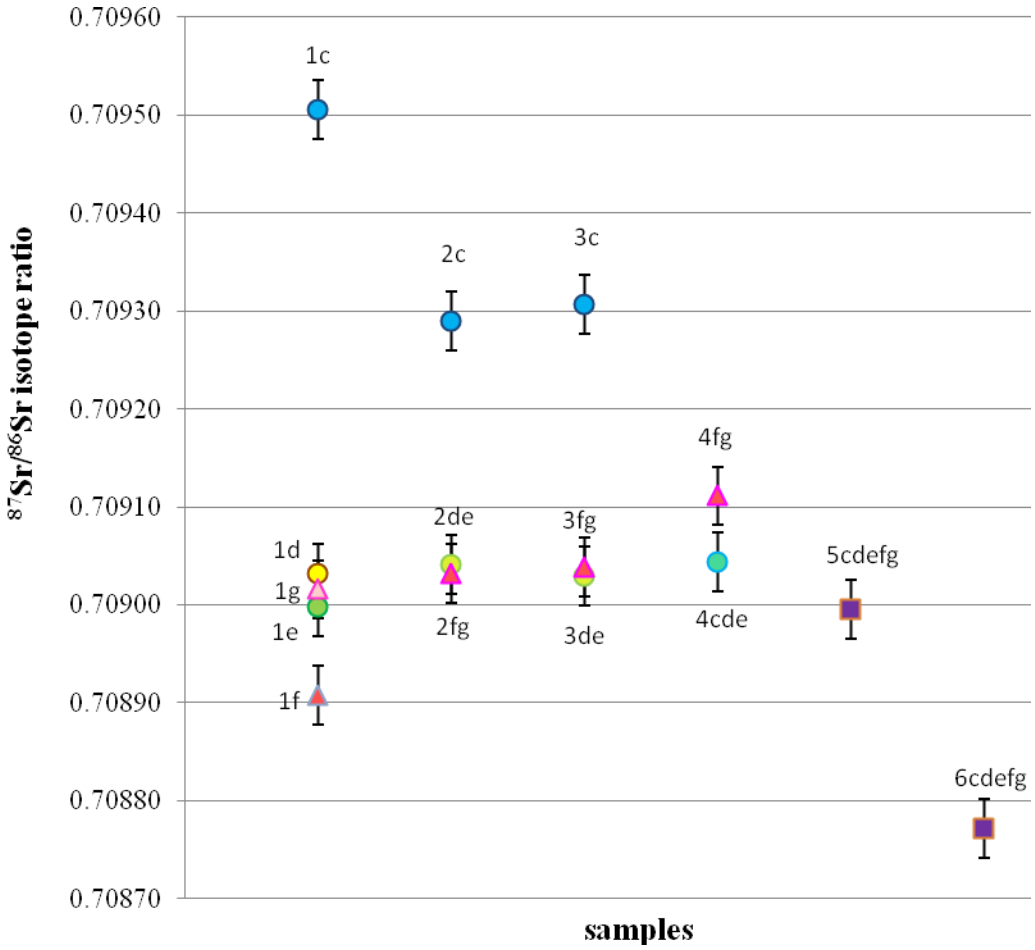

Figure 5.2. Graphical representation of the $^{87}Sr/^{86}Sr$ isotope ratios, measured for the second batch of CM winery. The error bars refer to the uncertainty associated to the measurement (± 0.00003).

As regards the first winery, PB, samples are depicted in blue in Figure 5.1 and the relative $^{87}Sr/^{86}Sr$ values are reported in Table 5.11.

In this case the situation is quite simple; the production comprises two starting silos of grape juices (PB1a and PB1b samples), which present different Sr-I.R. values, since grapes can come from various fields of the producer. In the second step, these samples are mixed obtaining a value which is perfectly in the range of the two starting juices, even if it is not the perfect average. This could be the consequence of mixing different amounts of the two samples. The following samples, from this intermediate to the final

commercial wine, present identical (differences below the measurement uncertainty) $^{87}Sr/^{86}Sr$ values.

Table 5.11. $^{87}Sr/^{86}Sr$ isotope ratios obtained for the samples of PB winery.

Sample code	$^{87}Sr/^{86}Sr$
JAG_PB1a	0.70916
JAG_PB1b	0.70888
JAG_PB2ab	0.70909
JAG_PB3ab	0.70908
JAG_PB4ab	0.70906
standard deviation*	0.00001

* standard deviation referred to samples 2ab, 3ab and 4ab.

The results concerning MO winery are reported in Table 5.12 and represented in green in Figure 5.1. The value relative to sample MO4 is not yet available, since the commercial wine was recently bottled.

Table 5.12. $^{87}Sr/^{86}Sr$ isotope ratios obtained for the samples of MO winery.

Sample code	$^{87}Sr/^{86}Sr$
JAG_MO1	0.71006
JAG_MO2	0.71002
JAG_MO3	0.70989
JAG_MO4	data not available
standard deviation	0.00009

The standard deviation of the three collected samples is slightly higher than the measurement uncertainty, but some consideration should be done. The Sr-I.R. values measured for the samples concerning the first two steps present no significant differences. A decrease in the $^{87}Sr/^{86}Sr$ ratio is found for the last sample, probably as a consequence of the addition of clarifying agents (bentonites, pvpp, gelatins), which

produce an extraneous Sr contribute. In this case, information about the $^{87}Sr/^{86}Sr$ values of the additives and the used amount could have helped to better understand the influence on the winemaking process.

In Table 5.13 and Table 5.14, the $^{87}Sr/^{86}Sr$ isotope ratio obtained for the three CE and CR batches are reported. The same are graphically represented in red, violet and yellow, respectively, in Figure 5.1.

Table 5.13. $^{87}Sr/^{86}Sr$ isotope ratios obtained for the two CE batches.

Sample code	**$^{87}Sr/^{86}Sr$**
First CE batch	
JAG_CE1a	0.70939
JAG_CE2a	0.70940
JAG_CE3a	0.70938
standard deviation	0.00001
Second CE batch	
JAG_CE1b	0.70929
JAG_CE2b	0.70930
JAG_CE3b	0.70917
standard deviation	0.00007

Table 5.14. $^{87}Sr/^{86}Sr$ isotope ratios obtained for the CR batch.

Sample code	**$^{87}Sr/^{86}Sr$**
JAG_CR1	0.709284
JAG_CR2	0.709293
JAG_CR3	0.709244
JAG_CR4	0.709241
JAG_CR5	0.709235
JAG_CR6	0.709219
standard deviation	0.00003

The Sr-I.R. value is perfectly transferred from grape juice to the intermediate products and then to the final product for the first CE batch and the CR production. A discrepancy in the final step of the second CE batch is revealed, which presents a significantly lower Sr-I.R. value. From the collected information, no explanation of this behavior can be suggested.

The results obtained for the first batch of CM winery are represented in orange in Figure 5.1 and reported in Table 5.15. The two starting silos of grape juice (CM1a and CM1b samples) present quite similar Sr-I.R. values. During the winemaking procedure, no significant variability in the strontium isotope ratio is found (standard deviation is equal to 0.00003). The addition of filtered must of Sorbara grapes (PAG_CM1) does not introduce changes, since the measured Sr-I.R. is not significantly below the values monitored in the intermediate products. As a matter of fact, the grapes used to obtain PAG_CM1 come from approximately the same area of those used for the produced wine.

Table 5.15. $^{87}Sr/^{86}Sr$ isotope ratios obtained for the first CM batch.

Sample code	**$^{87}Sr/^{86}Sr$**
JAG_CM1a	0.70867
JAG_CM1b	0.70872
JAG_CM2ab	0.70872
JAG_CM3ab	0.70868
JAG_CM4ab	0.70868
JAG_CM5ab	0.70866
JAG_CM6ab	0.70873
standard deviation*	0.00003
PAG_CM1	0.708646

* standard deviation referred to samples 2ab, 3ab, 4ab, 5ab and 6ab.

As regards the second CM batch (Figure 5.2 and Table 5.16), the situation is quite complex, since five starting silos of Lambrusco Grasparossa grape juice are mixed

together at different steps of the winemaking chain. The knowledge about the amounts of the mixed products is not so precise and detailed. As a consequence, no definite considerations can be drawn for all the production phases.

Table 5.16. $^{87}Sr/^{86}Sr$ isotope ratios obtained for the second CM batch.

Sample code	87Sr/86Sr
JAG_CM1c	0.709506
JAG_CM1d	0.709032
JAG_CM1e	0.708998
JAG_CM1f	0.708907
JAG_CM1g	0.709016
JAG_CM2c	0.709290
JAG_CM2de	0.709042
JAG_CM2fg	0.709032
JAG_CM3c	0.709307
JAG_CM3de	0.709030
JAG_CM3fg	0.709039
JAG_CM4cde	0.709044
JAG_CM4fg	0.709111
JAG_CM5cdefg	0.708995
JAG_CM6cdefg	0.708772
PAG_CM2	0.708533

The $^{87}Sr/^{86}Sr$ isotope ratios of the five starting samples are quite different, in particular sample CM1c at relatively high values. The obtained values for the samples concerning steps 2, 3 and 4 are intermediate among those of the five starting juices. CM2c and CM3c are at lower values than the respective CM1c sample, probably because those products are mixtures of samples CM1c, CM1d and CM1e. The same situation can occur for the other intermediate products.

Then, samples CM4cde and CM4fg are mixed to obtain CM5cdefg, with the addition of 5% of PAG_CM2. The Sr-I.R. value of PAG_CM2 is noticeably lower, but, due to the

small quantity added to the product, the decrease of the strontium isotope ratio of CM5cdefg with respect to the average of the two mixed samples is slightly appreciable. Finally, the wine (CM6cdefg) presents a considerably smaller Sr-I.R. than the sample of the preceding step. Because of the dimension of the winery, probably more production batches are blended to obtain the commercial product.

5.4.2. *TrentoDoc* winemaking chains

$^{87}Sr/^{86}Sr$ isotope ratio was determined on the grape juices and intermediate products of the investigated *TrentoDoc* winemaking chains. As for the previous samples, a measurement uncertainty of ± 0.00003 is associated to each value, calculated as twice the standard deviation of a replicated sample.

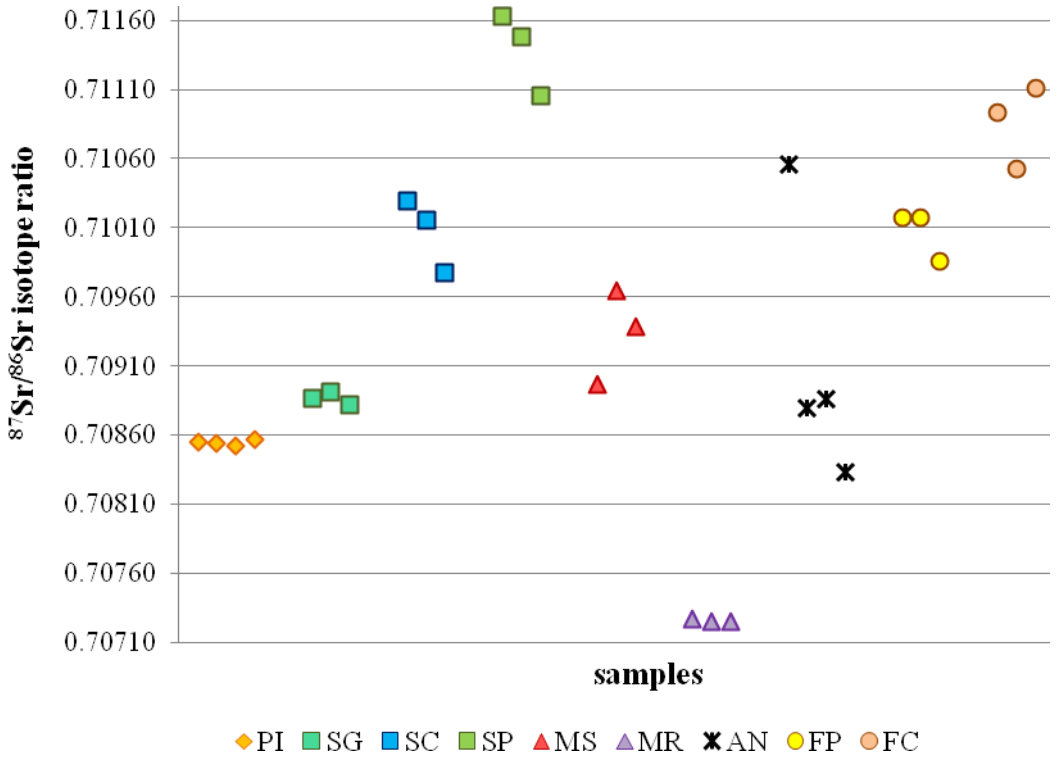

Figure 5.3. Graphical representation of the $^{87}Sr/^{86}Sr$ isotope ratios, measured for the monitored *TrentoDoc* productions. Symbol widths refer to the uncertainty associated to the measurement (± 0.00003)

The data obtained for the nine batches were graphically represented in Figure 5.3 and reported in the following tables.

Table 5.17. $^{87}Sr/^{86}Sr$ isotope ratios obtained for the first *TrentoDoc producer*.

Sample code	**$^{87}Sr/^{86}Sr$**
JAG_PI1	0.70855
JAG_PI2	0.70854
JAG_PI3	0.70853
JAG_PI4	0.70857
standard deviation	0.00002

Table 5.18. $^{87}Sr/^{86}Sr$ isotope ratios obtained for the second *TrentoDoc producer*.

Sample code	**$^{87}Sr/^{86}Sr$**
SG batch	
JAG_SG1	0.70886
JAG_SG2	0.70892
JAG_SG3	0.70882
standard deviation	0.00005
SC batch	
JAG_SC1	0.71029
JAG_SC2	0.71015
JAG_SC3	0.70978
standard deviation	0.00027
SP batch	
JAG_SP1	0.71163
JAG_SP2	0.71148
JAG_SP3	0.71106
standard deviation	0.00030

Table 5.19. $^{87}Sr/^{86}Sr$ isotope ratios obtained for the third *TrentoDoc producer*.

Sample code	**$^{87}Sr/^{86}Sr$**
MS batch	
JAG_MS1	0.70896
JAG_MS2	0.70964
JAG_MS3	0.70939
standard deviation	0.00034
MR batch	
JAG_MR1	0.70727
JAG_MR2	0.70725
JAG_MR3	0.70725
standard deviation	0.00001

Table 5.20. $^{87}Sr/^{86}Sr$ isotope ratios obtained for the fourth *TrentoDoc producer*.

Sample code	**$^{87}Sr/^{86}Sr$**
JAG_AN1	0.71056
JAG_AN2	0.70880
JAG_AN3	0.70887
JAG_AN4	0.70834
standard deviation	0.00097

Table 5.21. $^{87}Sr/^{86}Sr$ isotope ratios obtained for the fifth *TrentoDoc producer*.

Sample code	**$^{87}Sr/^{86}Sr$**
FP batch	
JAG_FP1	0.71018
JAG_FP2	0.71017
JAG_FP3	0.70986
standard deviation	0.00018
FC batch	
JAG_FC1	0.71094
JAG_FC2	0.71053
JAG_FC3	0.71111
standard deviation	0.00030

Unlike the Modena district, the $^{87}Sr/^{86}Sr$ isotope ratio was not previously determined on other matrices (soils, branches, wines, etc.) of the *TrentoDoc* production area; hence a direct comparison between strontium isotopic variation in soil and food cannot be made. From these results, it is evident a great variability of the values obtained for the different winemaking chains, which range from 0.7072 to 0.7116.

Looking at the Sr-I.R. trend along each winemaking chain, it is possible to notice opposite situations. If on one hand, the production process seems not to influence the $^{87}Sr/^{86}Sr$ value for three batches (PI, SG and MR), which present standard deviations below the measurement uncertainty, for the remaining productions is otherwise, with standard deviation reaching the fourth decimal digit.

Nevertheless, these results are preliminary. The reliability of the data should be verified, since the strontium concentration of most of the analyzed solutions was closer to the detection limit of the instrumental technique. For this reason, an alternative analytical procedure is under investigation, considering a different pretreatment of the sample (concentration of the sample on the resin) or using an alternative measurement system (desolvation nebulizer).

5.5. Conclusions

The use of $^{87}Sr/^{86}Sr$ isotope ratio as geographical tracer of food origin is strictly linked to its ability to transfer from the soil to the plant and then into the final product, maintaining unaltered values.

Studies on the features of this indicator suggest that, because of the small relative mass differences of the isotopes, the effect of isotope fractionation is not significant. As a consequence, all the transformations and biological processes which occur during the winemaking chain (i.e. fermentations, precipitations, etc.) should not affect the Sr-I.R. value. However, the cellar practices usually comprise the use of additives, such as clarification or deacidification agents as well as filtered or concentrated musts, or materials (cellar equipments, filtration tools, etc.), which could release a certain amount of strontium. Thus, the $^{87}Sr/^{86}Sr$ isotope ratio found in the final product is influenced by the winemaking process, depending on the amount and isotope ratio of the extraneous sources of strontium.

In this chapter, the influence of the winemaking process on the transfer of $^{87}Sr/^{86}Sr$ isotope ratio from the grape juices to the final product was investigated, taking into account seven *Lambrusco PDO* and nine *TrentoDoc* productions.

The obtained results confirm the assumption that the wineries use different vinification procedures, even if the same wine production is concerned, leading to different trend in the isotope ratio.

For most of the investigated winemaking chains, the Sr-I.R. values obtained for all the samples are in good agreement, showing no changes during the production and thus the possibility to directly relate the final product to its raw materials. In these cases, the addition of extraneous products is probably limited or, otherwise, products with the same geographical origin are added, as for the first CM batch.

On the other hand, some productions present variations of the isotope ratio. This variability in the behavior of the winemaking chains highlights the need to perform tailored investigation in order to identify the peculiarities of each process.

Further, a more detailed knowledge of the production procedures could allow a better understanding of the data, obtaining reliable information about the influence of different sources on the strontium isotope ratio along the winemaking chain.

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CHAPTER 6

Final remarks

The recent threats to food quality and safety have contributed to decrease consumer confidence in this field and to highlight the need for a better and more reliable system to trace the aliments and control their provenance.

Besides, consumers are more attracted by typical food products with a well declared origin or a link with a particular area, because they are felt as synonym of quality.

To protect and valorize this kind of foodstuffs, the European Community introduced the quality marks, namely Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Speciality Guaranteed (TSG). However, the increase in adulteration and counterfeiting phenomena has caused losses for the producers as well as risks for the consumers.

These issues are particularly relevant for a country like Italy, whose agro-food sector represents a milestone of utmost relevance, thanks to a great variety of well-known and high quality food products.

In this context, the definition of objective criteria to trace the origin of food has gained more and more interest, in order to obtain reliable tools for supporting the current traceability systems (RFID, bar and alpha-numerical codes, etc.) and the paper documentations.

The leading topic the work is the development of an analytical approach for the geographical traceability of food products.

Primary indicators, in particular the $^{87}Sr/^{86}Sr$ isotope ratio, are used to assess the food provenance, since they can be measured in all the matrices of the food production chain, including the soil, and values are found to be constant or varying according to precise relations.

The possibility to verify the cause – effect relationship between food and their territory of origin, which is the basis of the traceability models, strictly hinges on the accurate knowledge about the features of the investigated area and the behavior of the monitored indicators.

For these reasons, a systematic approach to the traceability issue and the use of $^{87}Sr/^{86}Sr$ isotope ratio for this purpose is proposed.

As a first step, the potentialities of a fast and simple screening technique were investigated in order to monitor the soil variability and features. The use of chemometrics tools to extract useful information from X-ray diffraction data, without the need to quantify each crystalline or amorphous phase, resulted crucial in order to improve the knowledge of the investigated area. The obtained results are helpful for the selection of the suitable sampling procedure or to reduce the number of samples to be analyzed by means of more expensive techniques, in the event of limited variability.
Moreover, when unknown areas have to be investigated, a study by using this method could help obtaining preliminary information.
Then, the trend of the $^{87}Sr/^{86}Sr$ indicator was studied, taking into account the transfer process from the soil to the grapevine, the grapes and all the steps of the winemaking chain. As a matter of fact, traceability studies are based on the guiding principle that the peculiar strontium isotopic signature of the soil could be transferred to the plant and then up in the food chain and production process till the final product, without further modifications due to isotope fractionation.
However, the $^{87}Sr/^{86}Sr$ of the final product can be influenced by the presence of different sources of strontium other than from the local bedrock geology as well as by the "selective" uptake of the plant.
The results of the pilot studies conducted on different regions show that the $^{87}Sr/^{86}Sr$ isotope ratio monitored in the bioavailable fraction extracted from soils is generally in accordance with that of the respective grape juices. Nonetheless, some areas present a great variability and the soil structure and composition are so complex that the NH_4NO_3 extractant results not perfectly able to reproduce the plant uptake. In these cases, the possibility to obtain data relative to the strontium isotope ratio measured in the plants (i.e. vine branches) is of great support. Actually, the use of the plant as a direct sampling device allows overcoming the issues about how to sample the soil matrix and how to analytically determine the real uptake. Further, the averaging action of the plant allows reducing the data dispersion, thus enhancing the discrimination of the samples according to their geographical origin. Moreover, the use of the plant as "sampling device" has some advantages with respect to the direct soil sampling. In fact, in the

former case, the vine has a direct access to a complex reservoir of nutrients through the roots. In any case, soil sampling may be more representative of that carried out by the plant itself.

A further phase which can alter the original $^{87}Sr/^{86}Sr$ isotope ratio through the introduction of external sources of strontium is the winemaking procedure.

Furthermore, two case studies were investigated, since the peculiar cellar practices can lead to different behaviors. The results highlight the need to carefully monitor the production phases obtaining detailed information about the treatments and the added products in order to establish if Sr-I.R. variations are expected. In the absence of extraneous sources of strontium, generally from additives, the $^{87}Sr/^{86}Sr$ isotope ratio should maintain the same value from the vine to the final products.

The analytical data reported in this thesis show an excellent potentiality of the strontium isotope ratio as traceability tracer. An excellent correlation is found between the values monitored in grape juice samples and the ones of their respective soils of provenance, as well as between the wines and the grape juices from which they are produced. Therefore, this research contributed to lay the foundations for the development of a large-scale traceability model, which will be obtained by considering representative sets of soils, branches and food matrices. The final model should be able to produce an isotope database for the authentication of the investigated wines and their discrimination from other similar products coming from outside the investigated territory on the basis of the monitored indicator.

APPENDIX 1

Use of X-ray diffraction technique and chemometrics to aid soil sampling strategies in traceability studies

L. Bertacchini, C. Durante, A. Marchetti, S. Sighinolfi, M. Silvestri and M. Cocchi, *Talanta*, vol. 98, pp. 178 - 184, 2012.

Finally I succeeded in this endeavor and I have to thank a lot of people, whose support was essential during these years (and not only).

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And what can I say about the rest of my colleagues/office mates/friends?? Well, this is a problem…Every word, I know, would be wrong or at least misunderstood. "Lista nera" docet! (By the way, I'm waiting for the Latin translation…).

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"One never notices what has been done;

One can only see what remains to be done."

Marie Curie