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La tracciabilità dei prodotti agroalimentari mediante la loro caratterizzazione chimica in termini di elementi in traccia (terre rare) e rapporti isotopici.

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Titolo della tesi: La tracciabilità dei prodotti agroalimentari mediante la loro caratterizzazione chimica in termini di elementi in traccia e rapporti isotopici.

Abstract:

In Italia il mercato dell'agro-alimentare è di primaria importanza ed è fondamentale sviluppare strategie che permettano di proteggere e valorizzare prodotti dalle caratteristiche uniche. Tali strategie si avvalgono anche del fondamentale supporto della chimica analitica.

Lo scopo del progetto di dottorato era sviluppare metodologie analitiche finalizzate all'identificazione di markers elementari e isotopici che permettessero di stabilire una relazione tra un prodotto agroalimentare e il suo luogo produzione o la tecnica di lavorazione. Nel corso del lavoro di ricerca sono state sviluppate e ottimizzate tecniche analitiche di spettrometria di massa applicate all'analisi elementare di campioni quali formaggio, orzo e malto. Gli studi di tracciabilità sono stati approfonditi sui campioni di orzo e malto, per i quali è stata ottimizzata anche una metodica analitica per l'analisi dei rapporti isotopici dello Sr. I profili multi-elementari ed isotopici ottenuti sono stati indagati mediante tecniche statistiche chemiometriche, che hanno permesso di discriminare i campioni sulla base dei loro siti di coltivazione, mettendo in evidenza una connessione diretta tra il luogo di coltivazione e la composizione del cereale stesso.

In Italy food market is of primary importance and it is fundamental to develop strategy to protect and appraise unique products. These strategies must pass also trough analytical chemistry support.

The aim of the PhD's project was to develop analytical methods to identify elemental and isotopic markers in order to establish a link between alimentary products and their production place or manufacturing techniques. Mass spectrometry analytical techniques were developed and applied for elemental characterization of cheeses samples, and barley and malt samples. Traceability studies were extended on barley and malt samples, optimizing the analytical technique for Sr isotope ratio measurements. Multi-elemental profiles obtained by instrumental analysis were investigated by means of statistical and chemometrics techniques. The investigation led to samples discrimination on the basis of their growing site, highlighting a connection between malt, barley and the cultivation site.

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Part I

General

Chapter 1

Introduction

The term traceability literally means "the ability to discover information about where and how product was made" (Cambridge dictionary definition). The food and feed production chain often involves many steps, from the import or primary production of a product to its sale to the final consumer. The production environment leaves a specific chemical fingerprint on products. By means of analytical chemistry, it is possible to investigate chemical composition of food and feed, and identify those traces. The aim of this study is to develop mass spectrometer methods that allow identification and quantification of elemental and isotopic composition of food-matrix and to apply them for the investigation of real alimentary products. The main goal is to identify specific elemental and isotopic markers that could define a specific connection between the foodstuff and the geographical area of production or a particular manufacturing technique. To achieve this purpose, the data obtained from samples analysis have to by studied by means of statistical and chemometrics instrument that can point out the correlation between the food composition and its origin.

In Italy food market is of primary importance and it is fundamental to be able of developing strategy to protect and appraise unique products. Those strategies must pass also trough analytical chemistry support.

Two different matrixes were taken in to exam during the last tree years, and they will be presented in two different section. My PhD research started with the study of Asiago Cheese traceability. After the first results the research was left in stand-by and the attention was moved to cereals, focusing on malt-type barley.

Foodstuff market reached a global scale in the last decades. Food and beverage coming from all-over the World can be easily bought at the supermarket. Consumers are increasingly concerned about the origin of foods they buy and they eat, they show increasing interests on organic and health foods, preferring the ones with a strong local identity. This is why food authentication and traceability are emerging topics involving people and authority. Food and

drink industry has an important role in the world economy. In Europe sales of food and drink products reached a turnover of 1,017billion euros in 2011, with an increasing of 6,8% compared to 2010. This economic field employee 2,25 million of people, and is one of the most grown. Concerning Italy, the turnover in 2011 is 31,8 billion euros, and it have been registered an increasing of +5,4% compared to the 2010 . On the basis of prevision, this data are going to increase in the next period. Due to the strong impact on the economy, institution and producer are very interested on this sector.

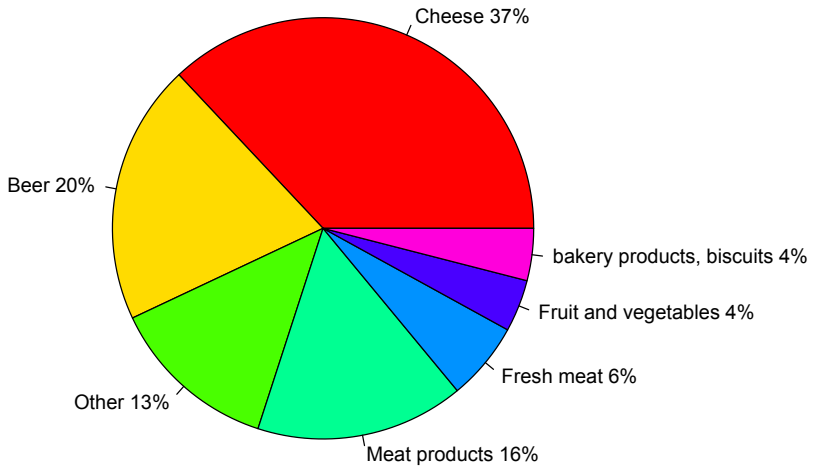


Figure 1.1: Percentage of total turnover for PDO/PGI agricultural products.

On consumers' side, there is an increasing interest for high quality foods, with a specific territorial identity. The reason is linked to many different aspects. People is searching for specific culinary, organoleptic qualities in food, health benefits is associated with this products. Consumers are concerned about animal welfare and 'environmentally friendly' production methods. There is also a kind of patriotism in the research for local food and beverage. Everything is linked with a decreasing confidence in the quality and safety of foods produced outside their local region or country. Public sensitivity has been touched by disease linked to food, like BSE[1] or the H7N9 [2] infection Also frequent food fraud, due to the presence of chemicals above acceptable limits, or

[1]Bovine Spongiform Encephalopathy.
[2]Influenza virus A subtipe, also known as avian influenza

not declared in feed and food, sometimes due to malpractices of food producers or sellers, can threaten both the quality and safety of products, endangering consumers health.

Starting from 1992 with the publication of the Council Regulation, than after, with new regulations and reviews, European Commission established legal rules, drawing the general principles and requirements of food law and procedures in matters of food safety. This policy framework is applied to the safety of food and feed circulating. It establishes rules for controlling and monitoring the production, prevention and management of risks. It also creates the European Food Safety Authority (EFSA), which is the reference point for the scientific control and evaluation of food and feed. With this outline, EU makes traceability compulsory for all food and feed business. Operators must be able to identify the origin of their products, and where there are going. The food and feed production chain often involves many steps, from the import or primary production of a product to its sale to the final consumer.

Tree specifics quality schemes were created from European commission to promote agricultural products, ensuring their authenticity: Protected designation of origin, Protected geographical indication and Traditional speciality.

Protected designation of origin - PDO -This status is given only to those products entirely manufacted within specific geographical area, using official production procedure. Italian Asiago cheese and Greek Kalamata olive oil are PDO products.

Protected geographical indication – PGI - This label is given to those products at least partially manufacted within a specific region. Red chicory of Treviso and Tiroler speck are PGI products.

Traditional speciality - TSG - It does not certify the link to a specific area but creates an exclusive right on the registered product name, that can be used only from producers that follow a specific production method, and ensures specific features. Italian mozzarella and Belgian Lambic beer are TSG products.

On a total of over 870 registered and labelled products, cheeses and beers together reach more

than 57% of the total turnover. European Commission in 2008 registered a value of more than 14,2 billion euros for PDO/PGI products that for the most is powered by cheese (5,6 billion euros) and beer (2,3 billion euros) commerce. On this scenario, analytical chemistry plays a relevant role on food authentication, reflected in scientific field with an increasing number of published research articles. Scientific research support EU efforts, developing analytical techniques capable to identify specific markers related to the geographical origin, but also related to food degradation or food adulteration.

Chapter 2

Analytical chemistry in food authentication

Analytical chemistry is a powerful instrument in the field of food authentication. Many different analytical approaches are used to identify and quantify traces that connect a product to a particular manufacting procedure, or a specific geographic area. The analysis of organic components in foodstuff is not suitable in tracing procedures because organics are prone to huge variability. Nonetheless some authentication research was published. The investigation of elemental composition and isotopic composition in food and beverages is more suitable for authentication purpose because they provide unique fingerprint.

2.1 Elemental composition

Multi-element composition of foodstuff reflects in some extent the composition of the environment in which the product has grown up[**Pro**, **Cheajesadagul2013**]. For meat, for example, the multi-element composition reflects that of the vegetation that the animal eats[**ArgMeat**]. The same can be said for milk, and dairy products[**Pillonel2003**]. In the final product there is the environmental fingerprint. Plant species reflect the composition of the bio-available and mobilized nutrients present in the soils where they grown up. Elements in the soil can be mobilized, and then transported into plants, so they become a good indicators of geographical origin. Several factors affects trace element availability such as humidity, soil pH, humic complex etc. This means that elemental composition gives unique markers in food and characterizes geographical identity [**Kelly2005**].

2.1.1 Rare Earth Elements

A total of 17 elements are referred to as the rare earth elements. Lanthanide elements have atomic numbers ranging from 57 to 71 (La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu), with the inclusion of scandium (Sc) and yttrium (Y). "Rare" heart element is an improper definition, considering that, with the exception of Pm that is unstable, their concentration is quite high in earth's crust. La, Ce and Nd are more abundant than Pb. In nature lanthanides are mainly present as oxide. Elements from Sc to Gd are called light rare earth elements (LREE), Y, Tb, Dy, Ho, Er, Tm, Yb, and Lu are also known as heavy rare earth elements (HREE). Un nature REE represents the prime example of the Oddo-Harkins Rule. Even numbered elements are more abundant than odd numbers elements.

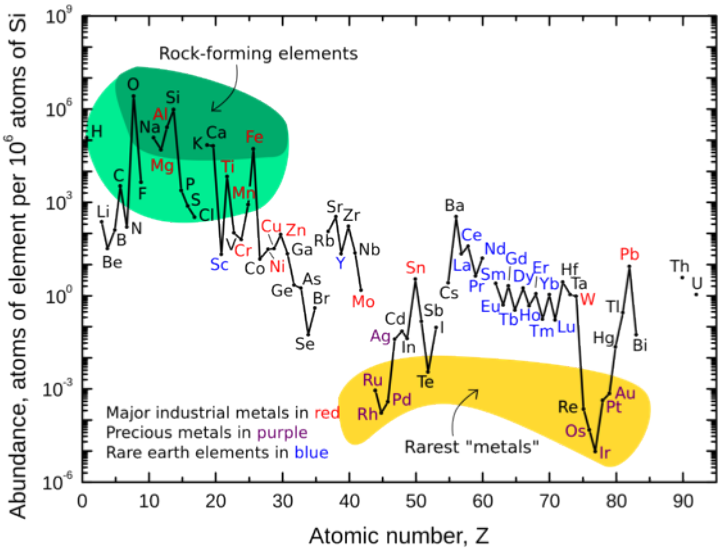


Figure 2.1

Name	Symbol	Atomic Number	Stable Isotopes (mass numbers)	Earth's crust abundance ($\mu g g^{-1}$)
Scandium	Sc	21	45	25
Yttrium	Y	39	89	31
Lanthanum	La	57	139	35
Cerium	Ce	58	140	66
Praseodymium	Pr	59	141	9.1
Neodymium	Nd	60	142, 143, 145, 146, 148	40
Promethium	Pm	61	–	$4.5 \cdot 10^{-20}$
Samarium	Sm	62	144, 149, 150, 152, 154	7
Europium	Eu	63	151, 153	2.1
Gadolinium	Gd	64	154, 155, 156, 157, 158	6.1
Terbium	Tb	65	159	1.2
Dysprosium	Dy	66	156, 158, 160, 161, 162, 164	4.5
Holmium	Ho	67	165	1.4
Erbium	Er	68	162, 164, 166, 167, 168, 170	3.5
Thulium	Tm	69	169	0.5
Ytterbium	Yb	70	168, 170, 171, 172, 173, 174, 176	3.1
Lutetium	Lu	71	175	0.8

Rare earth elements have a very high similarity in their chemical properties, therefore they usually occur jointly. They have similar ionic radii, decreasing with the enhancement in atomic number; this phenomenon is called lanthanide contraction. The most of rare earth elements have single 3+ valence, with some exception. This characteristic imposes limitation in their fractionation. Ce and Eu can occur in different oxidation states and often show greater fractionation relative to the other REE. Lanthanide concentration in soils can vary a lot, and their bioavailability varies with pH, organic matter, weathering state. Transfers factor soil/plant are very low, and it was observed a decreasing in concentration from soil to roots, up to fruits. However it was observed that REE pattern in plants is related to the REE soil content. For this reason Lanthanides can be used as provenance indicator. They have been used to identify geographical origin of oil(Joebstl et al., 2010) (Benincasa et al., 2007), tomatoes [**Spalla2009**]. Also wine traceability was studied, but producing processes should be taken into consideration, because it can affect the REE content[**Aceto2013**] (jakubowski et al., 1999)(Aceto et al., 2013). Accurate quantitative measurement of rare earth elements can be performed wit mass spectrometry, but is not a trivial matter. Rare earth element abundances are usually normalized before being compared for interpretation. Concentration values are corrected to a common baseline and the result of normalizing is comparing how much groups vary from a common starting point rather than the final magnitude of the groups. Normalization is accomplished by dividing the abbundance of the measured samples by the abbundance of a choosen standard. In this way the effect due to the Oddo-Hakins Rule on the concentration, that is reflected in a herringbone-shaped curve, is smoothed, allowing a better comparison[**Piper2013**]. There are several geological standards that can be used so as to normalize the REEs, like World Shale Average (WSA), the North American Shale Composite (NASC), Post Archaen Australian Shale (PAAS), the Upper Continental Crust (UCC) and the Chondrites [**Piper2013**] (Nozaki)

2.2 Isotopic composition

Many natural phenomena can lead to measurable changes in the ratio of the 'heavy' to 'light' isotope of a given element. Isotopic fractionation is due to evaporation and condensation, crystallisation and melting, absorption and desorption, diffusion and thermo-diffusion, bacterial activities, etc. Determination of isotope ratios can be used for provenance study. Hydrogen and oxygen isotopic ratio have a very strong reliance on latitude, so they are good geographic indicators. Carbon and nitrogen isotope ratios can give information about plant sort or animal diet and sulphur isotopic composition can be related to the weathering of bedrock, but also to microbial processes in soils, and marine activity.

2.2.1 Strontium isotopic composition

Strontium is an alkaline earth metal, and ^{84}Sr,^{86}Sr, ^{87}Sr and ^{88}Sr are his four natural stabiles isotopes. Its ionic radius is similar to that of calcium and strontium can substitute for calcium

Isotope ratio	Fractionation	Information
$^{2}H/^{1}H$	Evaporation, condensation, precipitation	Geographical
$^{13}C/^{12}C$	C3 and C4 plants	Diet (geographical proxy)
$^{!5}N/^{14}N$	Trophic level, marine and terrestrial plants, agricultural practice	Diet (geographical proxy)
$^{18}O/^{16}O$	Evaporation, condensation, precipitation	Geographical
$^{34}S/^{35}S$	Bacterial	Geographical (marine)and Taylor
$^{87}Sr/^{86}Sr$	Age of the rock and Rb/Sr ratio	Geological, geographical

in a wide range of naturally occurring minerals.

Element	Mass Number	Isotopic Abbungance
Sr	84	0.56
	86	9.86
	87	7
	88	82.58
Rb	85	72.17
	87	27.83

The isotope ^{87}Sr is formed by radiogenic decay of ^{87}Rb whit a half-life of $48.8 \cdot 10^{9}$years.

$$^{87}Rb \longrightarrow^{87} Sr + \beta^{-} + \nu \tag{2.1}$$

The ratio of the radiogenic nuclide to a reference isotope $^{87}Sr/^{86}Sr$ is used for geochronological purposes, the higher is the ratio the older is the rock. The amount of $^{87}Sr/^{86}Sr$ is used also as geological tracer. Strontium moves into soil and groundwater, and to plant, from bedrock without measurable fractionation, and its concentration depends on the local geology. Sr isotope ratio is a powerful instrument for provenance study. Birds' migration, population migration was reconstructed with the aim of Sr isotope ratio, and also food provenance study has been performed [**Swoboda2008**] As a result of their different mass, isotopes of an element can be separated from one another using mass spectrometry. The isotopic composition of the light elements H, C, N, O, and S is usually studied via gas source MS, and for 14C dating, accelerator mass spectrometry (AMS) is replacing radiometric techniques. Metals and metalloids isotopic analyses are usually performed by thermal ionization mass spectrometry (TIMS) and multicollector (MC)-ICP-MS.

2.3 Mass Spectrometry

Inductively coupled plasma techniques are the most widely used in analytical chemistry to perform multi-elemental and isotopic analysis. The wide range of concentration they can detect, and

their speed of analysis makes those techniques suitable for food analysis, in order to characterize them, to trace them and to investigate possible frauds. Inductively coupled plasma mass spectrometry (ICP-MS) is a suitable analytical technique for simultaneous determination of trace and ultra-trace analyte concentration, being able to quantify around 8 order of magnitude of concentration unit. Mass spectrometers are composed of an ion source a mass analyser and a detector.

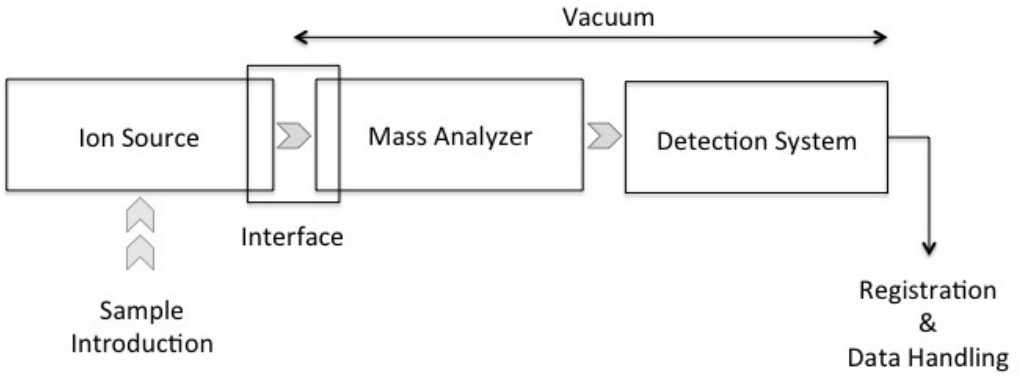


Figure 2.2: Mass spectrometer schematic

2.3.1 Sample introduction

Liquid samples are introduced in ICP-MS using a nebulizer that creates an aerosol. The sample is pumped into the nebulizer where the liquid is smashed in small droplets by the pneumatic action of a gas flow (usually argon). Than a spray chamber select the droplets, to let pass by only the smallest. The selection is due to the chamber geometry. The small drops reach the plasma for dissociation, atomization and ionization, the other are discarded, and pumped away. There are many different nebulizer and spray chamber designs, customized for specific application.

2.3.2 Ion source

In ICP-MS the ion source consists in inductively coupled plasma, sustained in a torch. The torch is composed by three concentric tubes (2.3) in which pass argon flow. At the end of the torch is placed an induction coil supplied with a radiofrequency electric current.

Argon ionization starts with a spark generated from the Tesla Coil. This strips away some electrons from the atoms. The ions generated and their electrons interact with the fluctuant electric field and forcing them to accelerate in a ring path, producing ohmic heating, proceeding to a chain reaction that feed the plasma with argon ions and electrons. In the torch outermost

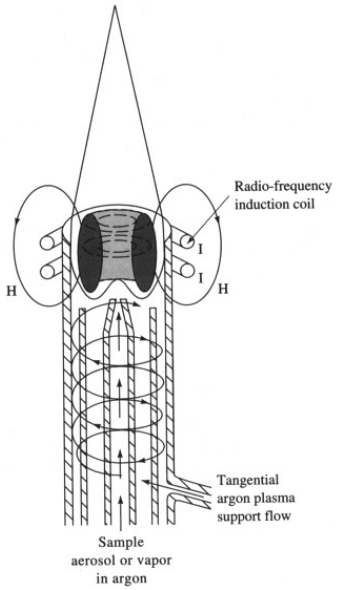


Figure 2.3: Plasma torch

tube flows the plasma or coolant gas. It is the gas that constitutes the plasma. It is introduced tangentially, and it leads to keep the plasma away from the sides of the torch, to preserve it from melting, and lead the centring of plasma. In the middle tube there is an auxiliary gas flow, which main function is to push the plasma away from the injector tube. The injector tube is the innermost tube in which the gas flow carries the sample. The ionization of the sample occurs because of a number of physical changes, in a mechanism that can be summarized as in 2.4.

$$M(H_2O)^+X^- \xrightarrow{Desolvatation} (MX)_n \xrightarrow{Vaporization} MX \xrightarrow{Atomization} M \xrightarrow{Ionization} M^+$$

Figure 2.4: Schematic mechanism of positive ion formation.

Argon is the chosen gas for making the plasma because is abundant and cheaper compare to other gas, and his first ionization potential is higher than the most of the elements. This ensure the preserving of ionized sample, because is more energetically favourite the reaction $Ar^+ + e^- \longrightarrow Ar$, instead of $M^+ + e^- \longrightarrow M$.

2.3.3 Interface

Between the ion source and the mass analyser there's an interface that has to connect a section at atmospheric pressure to the analyser at high vacuum. This region is delimited from two metallic cones throw that passes the ions. The first cone, the sampler cone, in the centre has a $1mm$ hole, and is brushed by plasma flame. It isolates the riches in ions part of the plasma, and let them pass by, trough the interface region, to the second cone, the skimmer cone that with a $0.4mm$

hole let pass by the ions. After cones region, inside the high vacuum region, ion lenses focus and collimate the ion.

2.3.4 Mass analyser

The mass analyser discriminates the ions depending on their mass to charge ratio. Different mass analysers, with very different performance, are used in this field.

The quadrupole filter is the most common mass filter. Is composed by four cylindrical rods, to which, in pairs, is applied a radio frequency (RF) voltage, and a superimposed current voltage. Ions travel across the quadrupole, and only the ones with a certain m/z value will reach the detector, on the basis of the applied voltage. The ions with a different ratio are discharged along the quadrupole length. Varying the applied voltages it is possible to obtain fast m/z scan. Quadrupole mass analyzers are basically operating at low resolution; they have indeed limitations in their resolving power, compared to other mass analysers.

Resolution is defined as the capability of a mass spectrometer to differentiate between masses (Nelms) and is calculated wit the equation:

$$R = \frac{m}{\Delta m} \tag{2.2}$$

Where m is the nominal mass of the analyte peak and Δm is the mass difference between two resolved peaks.

	Resolution Range	Resolution
Quadrupole	400	Low
Double focusing	$300 - 400$	Low
	$3000 - 4000$	Medium
	$8000 - 10000$	High

The combination of magnetic and electrostatic fields in mass discrimination, allows up to twenty time higher resolving power in double-focusing mass spectrometry, compared to quadrupole. Ion produced by plasma, are focused by lenses, and they pass trough a thin slit of adjustable width, before being injected perpendicularly into the magnetic sector analyser. The magnetic sector is composed of a curved tube, placed between the poles of a magnet. Passing trough the magnetic field the ions trajectory undergoes to a different flexing, according to their m/z ratio. To enhance the magnetic sector performance, an electrostatic analyser (ESA) can be insert prior or after the magnet. The electrostatic analyser is composed of two curved parallel plates to which is applied DC voltage. In single-collector magnetic sector field ICP-MS instruments the electrostatic analyser is placed after the magnetic sector field. In multi-collector instruments instead a 90 electrostatic analyser is combined with a 60 magnetic sector (known as Nier–Johnson geometry).

2.3.5 Detection system

After being separated on their m/z ratio, ions must be detected, and their signal must be amplified, in order to determine them. The detection system converts the ions in in to electrical pulse, which magnitude is related to the concentration of the single ion. So far different detection designs have been used, but nowadays, the most common are the discrete dynode electron multiplier. Basically, when an ion impact on the first dynode, a certain number of secondary electrons are released from his surface. Those electrons are accelerated to the second dynode, generating more electrons, and so on, until the pulse of electron is finally captured, and they are converted in a signal, to create a mass spectrum. Some instruments are equipped with Faraday cups as ions detector. In multicollector ICP-MS several faraday cup are placed within a collector block.

2.3.6 Interferences

A hurdle in mass spectrometry is given by isobaric interferences. Elemental ions or polyatomic ions due to the plasma gas, the matrix component, or the solvent-acid used, can overlap the mass of the analyte of interest, causing the increasing in detection limits and the decreasing in quantitative accuracy.

Analyte	Isobaric interference	Resolution
^{56}Fe	$^{40}Ar^{16}O$	11451
^{39}K	^{38}ArH	5690
^{80}Se	$^{40}Ar^{40}Ar$	112570
^{75}As	$^{40}Ar^{35}Cl$	28809
^{52}Cr	$^{40}Ar^{12}C$	5372
^{51}V	^{35}ClO	4648

To overcome this problem, several methods can be used, as acting on plasma temperature, but those methods are not easy to optimize. Usually, to minimize isobaric interferences in MS, the collision/reaction cell is used. Ions are extracted under vacuum from the interface region, to the collision/reaction cell, before entering the mass analyser. The cell consists in a multipole (quadrupole, exapole, octupole), in which is pumped in a suitable collision/reaction gas (e.g. He, H, Ammonia, etc.). The interferences are overthrown converting them in to harmless species, or by adding a mass to the analyte, moving to a free of interferences zone.

$$^{137}Cs^+ + ^{137}Ba^+ + N_2O \longrightarrow ^{137}Cs^+ + ^{137}BaO^+ + N_2 \text{ (Isobar shift)}$$
$$^{40}Ca^+ + ^{40}Ar^+ + ^{1}H_2 \longrightarrow ^{40}Ca^+ + ^{40}Ar + ^{1}H_2^+ \text{ (Charge exchange)}$$
$$^{87}Sr^+ + ^{87}Rb^+ + CH_3F^+ \longrightarrow ^{87}SrF^+ + ^{87}Rb^+ + CH_3 \text{ (Analyte shift)}$$

2.4 Sample preparation

Sample preparation is a fundamental step in analytical chemistry. Samples usually are not ready for direct analysis, so sample preparation has to be done. Samples processing can vary a lot depending on analytical technique used for the determination, on the concentration range of the analyte, and on the sample matrix to analyse. For this reason, preparation for elemental analysis can include different procedures, than can be summarized as:

- Matrix degradation and solubilisation to release all the analyte.
- Analytes extraction with a suitable solvent.
- Analyte concentration or dilution, to reach a suitable range for instrumental analysis.
- Separation of a single element or a group of elements, to reduce, for instance, the interferences in analiysis

Quantitative determination of elements in solids samples, usually implicate a preanalytical phase that lead the extraction of all the analyte of interest, into a solution. The most common way to obtain a solution containing the analytes of interest are acid digestion, and extraction.

2.4.1 Acid digestion

Acid digestion procedures are employed for solids samples preparation in order to completely transfer the analytes into solution so they can be introduced into the analytical instrument for the analysis. The goal of the digestion process is to completely dissolve the solid matrix, leading to the total solution of the analytes. To perform acid digestion concentrated mineral acids (e.g., HNO_3, HF, HCl, H_2SO_4, etc.) are usually used, sometime in combination with oxidizing agent (e.g. H_2O_2).

Wet chemical digestion can be performed in in open containers; some acid reagent is added to the sample and the digestion reaction take place under atmospheric pressure. Closed vessels systems are also used to dissolve the samples. The samples can also be heated with the aim of the hot plate (or other convection devices), or by microwave oven. The increasing of temperature supports the samples dissolution. If this is done in a closed vessel system, the reaction kinetics increases a lot, reducing the digestion time. Close vessels digestions reduce loss of analyte, and the risk of sample contamination, but the pressure increasing in the vessel can be a potential safety hazard. Sealed vessels are usually used in microwave acid digestion. The vessels, made of chemical inert material, are located in a rotor, inside the oven. A temperature probe is inserted in a vessel, so is possible to check the reaction temperature. Some digestion systems are also furnished with a pressure control. During the microwave digestion process, the rotor turns to ensure the continue stirring of the sample and the acid solution. A digital controller shows and registers all the digestion parameters.

2.4.2 Extraction

Sometimes is not necessary to evaluate the whole metal content in the sample, destroying it. The analyte of interest may be present in a soluble form, and we don't need to know the elemental composition of the whole solid matrix. An appropriate solvent is added to the sample in a container. The extraction can be supported usong ultrasonic bath, or by using a mechanical shaker. After extraction, the surnatant is usually divided from the solid matrix by filtration or using a centrifuge.

2.4.3 Preconcentration and sample purification

After total digestion or extraction, the result is a liquid sample. In the obtained solution the concentration of the analyte of interest can be under the instrumental detection limit. If the analyte concentration is too low, a preconcentration step is necessary. A hotplate can be used to warm-up the sample, producing the solvent evaporation. It is important not to exceed with the temperature, to avoid spattering, the solution should boil very gently, until reaching the desired volume. Solid phase extraction (SPE) is a separation process, used to concentrate metals (and other chemical species), acting on a specific element, or a specific class of analyte. In SPE extraction, the elements suspended in the solvent, flows trough a column, cartridge, or disc that contains a solid material that bind some specific class of analytes. Once all the solution is passed by, it is possible to collect the eluted fraction, or, discard it if we are interested in the elements bind to the resin. Washing the stationary phase with an appropriate eluent, it's possible to release the analyte of interest, free from interferents, and concentrated.

2.5 Statistic analysis

Even the simplest food is a very complex matrix, and the better way to investigate its chemistry is a multivariate approach. The large amount of instrumental data to be processed requires the use of statistical tools and specifically the use of chemometrics. Since the very beginning, chemometrics has been deeling with different problems related to food quality (6-8 da chemometrics food). Today chemometrics in food analysis and in particular in food traceability is not only recommended, it is essential. Infact no specific phisico-chemical markers have been identified that can be univocally linked to the origin of a foodstuff; the only way to obtain a reliable traceability is the use of multivariate classification to experimental data (CIT libro Chemometrics in food chemistry). Chemometrics includes many multivariate tools which can be usefull to manage experimental data. This work investigates data set using Cluster Analyis and Principal Component Analysis (PCA).

2.5.1 Cluster Analysis

Cluster analysis methods represent a family of chemometric tools alternative or complementary to the projection of latent variables such Principal Component Analysis and other techniques. The main target of cluster analysis is to find groups within a given data set, basing on the principle for which similar objects are represented by close points in the space of variables which describe them. Cluster analysis itself is not one specific algorithm, but the general task to be solved. It can be achieved by various algorithms that differ significantly in their notion of what constitutes a cluster and how to efficiently find them. Clustering can therefore be formulated as a multi-objective optimization problem. The appropriate clustering algorithm and parameter settings (including values such as the distance function to use, a density threshold or the number of expected clusters) depend on the individual data set and intended use of the results. Cluster analysis as such is not an automatic task, but an iterative process of knoledge discovery or interactive multi-objective optimization that involves trial and failure. It will often be necessary to modify data preprocessing and model parameters until the result achieves the desired properties. A Cluster Analysis starts from the data matrix X of size nxp and which is transformed into a nxn matrix of dissimilarity or distance between the n pairs of observations (vectors p elements). Then an algorithm has to be selected to defines the rules for how grouping the units into subgroups on the basis of their similarity. The purpose is to identify a smaller number of groups such that the elements belonging to a group are - in some sense - more similar to each other than to elements belonging to other groups. The fundamental starting point and the definition of a measure of similarity or distance between objects (i.e. between the rows of the data matrix). Another essential point is the rule according to which forming groups. Depending on the type of data, different measurements are used; quantitative data are managed using measures of the distances, instead qualitative data are managed using measures of association. The output of a cluster analysis is a graph called dendrogram that displays the stacking order

of objects examined. In essence it displays the whole process of aggregation that is a hierarchy of partitions. A single partition is obtained by "cutting" the dendrogram at a given level of the hierarchy's index of distance.

Here an extensive discussion of the theory of the Cluster Analysis will not be effort, but criteria used to perform the processing of experimental data will indicate. Cluster Analysis has been performed choosing the Euclidean distance as metric:

$$d_{i,h} = \sqrt{\sum_j (x_{ij} - x_{hj})^2}$$

the method used is a hierarchical aggregative clustering and Pearson linkage. Cluster analyses performed in this study were accomplished using the software Statistica 6 (Statsoft Italia s.r.l., Via Parenzo 3 - 35010 Vigonza-PD - StatSoft, Inc. 2300 East 14th Street Tulsa, OK 74104, USA).

2.5.2 Principal Component Analysis

PCA is a multivariate analysis based on Pattern Recognition, proposed for the first time by Karl Pearson in 1901 and developed by Harold Hotelling in 1933. PCA is a bilinear decomposition/projection technique capable of condensing large amount of data into few parameters, called Principal Components (PCs), which capture the levels, differences and similarities among the samples and the variables included in a data set. This statistical procedure uses a linear transformation preserving data variance and imposing othogonality of the latent variables (once uncovered, latent variables – PCs may be represented by scatter plots in a Euclidean plane). Thus a data set of correlated variables is converted into a set of uncorrelated principal components. This new dataset should best represent the original variance of the datapoints. The number of principal components is less than or equal to the number of original variables. This transformation is defined in such a way that the first principal component has the largest possible variance (that is, accounts for as much of the variability in the data as possible), and each succeeding component in turn has the highest variance possible under the constraint that it is orthogonal to (i.e., uncorrelated with) the preceding components. In PCA, the extractions of PC can be made using either original multivariate datasets or using the covariance or the correlation matrix if the original dataset is not available. In deriving PC, the correlation matrix is commonly used when different variables in the dataset are measured using different units or if different variables have different variances. Using the correlation matrix is equivalent to standardizing the variables to zero mean and unit standard deviation. A correlation matrix can be decomposed in eigen values/vectors. Eigenvalues measure the amount of the variation explained by each PC and will be largest for the first PC and smaller for the subsequent PCs. An eigenvalue greater than 1 indicates that PCs account for more variance than accounted by one of the original variables in standardized data. This is commonly used as a cutoff point for which PCs are retained. Eigenvectors provides the weights to compute the uncorrelated PC, which are the linear combination of the centered standardized or centered un-standardized original variables. ScreeTest Plotting

the eigenvalues against the corresponding PC produces a screeplot that illustrates the rate of change in the magnitude of the eigenvalues for the PC. The rate of decline tends to be fast first then levels off. The "elbow" or the point at which the curve bends, is considered to indicate the maximum number of PC to extract. One less PC than the number at the elbow might be appropriate if you are concerned about getting an overly defined solution. PC loadings are correlation coefficients between the PC scores and the original variables. PC loadings measure the importance of each variable in accounting for the variability in the PC. It is possible to interpret the first few PCs in terms of "overall" effect or a "contrast" between groups of variables based on the structures of PC loadings. High correlation between PC1 and a variable indicates that the variable is associated with the direction of the maximum amount of variation in the dataset. More than one variable might have a high correlation with PC1. A strong correlation between a variable and PC2 indicates that the variable is responsible for the next largest variation in the data perpendicular to PC1, and so on. If a variable does not correlate to any PC, or correlates only with the last PC, or one before the last PC, this usually suggests that the variable has little or no contribution to the variation in the dataset. Therefore, PCA may often indicate which variables in a dataset are important and which ones may be of little consequence. Some of these low-performance variables might therefore be removed from consideration in order to simplify the overall analyses. PC scores are the derived composite scores computed for each observation based on the eigenvectors for each PC. The means of PC scores are equal to zero, as these are the linear combination of the centered variables. These uncorrelated PC scores can be used in subsequent analyses, to check for multivariate normality, to detect multivariate outliers, or as a remedial measure in regression analysis with severe multi-collinearity. The investigation of the inter-relationships between the observations and variables in multivariate data is performed using a visualization technique called Bi-plot. To display a bi-plot, the data should be considered as a matrix, in which the column represents the variable space while the row represents the observational space. The term bi-plot means it is a plot of two dimensions with the observation and variable spaces plotted simultaneously. In PCA, relationships between PC scores and PC loadings associated with any two PCs can be illustrated in a bi-plot display. Principal components are guaranteed to be independent if the data set is jointly normally distributed. It is important to remember that PCA is sensitive to the relative scaling of the original variables. The calculations performed in this work where accomplished using the software Statistica 6 (Statsoft Italia s.r.l., Via Parenzo 3 - 35010 Vigonza-PD - StatSoft, Inc. 2300 East 14th Street Tulsa, OK 74104, USA).

Part II

Asiago cheese

Chapter 3

Parte generale

The aim of this work was to develop a suitable analytical method, able to point out difference and similarity in elemental composition on cheeses of different aging. To reach that goal, four different kind of Asiago cheese have been studied, first chemically, then statistically.

The work is based on the early assumption that place of production and food processing leave traces on final products[?][?][?]. With an appropriate analytical method development, specific for the matrix of interest, it is possible to evaluate the different traces due to the origin of the product and the technique of row material processing.

3.1 Introduction

In Italy milk-dairy is the first foodstuff division. This production has a turnover of 14.2 billion euros, that represents more than 12% of the total turnover due to the foodstuff trade market. In August 2013 the export of diary products registered a +6.1%, reaching 213000 tons of diary products made in Italy. A big part of the turnover is related to POD (protected designation origin) Those demonstrate that foodstuff commerce is a very significant division in Italian Economy. For this reason is important to protect the products, and the consumers. Analytical chemistry can held the goal, by developing methods that can identify and certify the genuinely and authenticity of foods. Studying the chemical fingerprint of cheeses can give an identity to the products, supporting authority to discover fraud, and to protect the consumers' health.

3.1.1 Asiago cheese

The Asiago cheese is an Italian cheese that takes his name from the Asiago Plateau, a vast highland located in the Alps in Vicenza, at the border between the regions of Veneto and Trentino-Alto Adige. Asiago cheese was produced since year 1000 and initially it was made with sheep's milk, but from the 1500s, cow's milk was used as raw material. The cheese producing technique developed along the time, and, during the early seventeenth century, production expanded to

the foothills, the surrounding plains and the nearby Alpine huts of Trentino, the surrounding areas of the Asiago Plateau. In 1979 the Consortium for the Protection of Asiago Cheese arises, with the aim of guarantees the traditional identity of Asiago chees, making sure that only cheese produced under specific rules could be called, and sold as Asiago. Milk producers, cheese makers and seasoners, and they take care to protect and promote the POD Asiago cheese. The protected designation of origin "Asiago" is reserved to cheese produced according to procedural guideline (Ministerial Decree 03/08/2006 Official gazette no. 190, 17/08/06). The guideline regulated the production area, the animal feed, the production procedure, the marking rules, the aging and storage methods, the characteristics of the finished product and also the packaging and labelling for the trade. Nowadays it is possible to find in commerce different kinds of Asiago cheeses.

- Fresh Asiago, (pressed Asiago) whose production started in the early twentieth Century, is seasoned at least 20 days. It has a milky taste, and it can be recognized because of his pale yellow colour, the marked and irregular holes and the soft and elastic consistency.
- Aged Asiago (fostered Asiago) is more compact and flavoured than Fresh one.The Aged Asiago can be recognized because of his yellow, pale-yellow colour, the small holes in the structure, and the hardness, that, as fore the taste, increases with the aging process.
  - Asiago "Mezzano" (medium seasoned), his seasoning last 4-6 months;
  - Asiago "Vecchio" (mature) who's seasoning last 10-15 months;
  - Asiago "Stravecchio" (extra mature) seasoned for over 15 months.
- Asiago cheese "Product of the Mountain". To produce this cheese it's used only mountain milk, chees transformation and maturation take place exclusively at an altitude of between 600 and 2300 metres, in the mountains of the DOP region between Veneto and Trentino.

Chapter 4

Experimental

Sample preparation, standard dilution and all the laboratory work has been performed in a class

4.1 Materials and Methods

Reagents and standards

- Nitric acid 65% Suprapur - Merck KGaA, Darmstadt, Germany
- Hydrogen peroxide 30% Suprapur - Merck KGaA, Darmstadt, Germany
- High-purity water 18.2 MΩ - Elga LabWater system, Veolia Water VWS, UK
- Multi elemental standard ICUS1675-
- Standard Reference Material (SRM) 8435 *Whole Milk Powder*

Laboratory ware

- 50 mL PE centrifuge tubes – Iwaki
- 15 mL PE centrifuge tubes – Iwaki
- PTFE Spatulas
- PS Sterile Tweezers
- Plastic Grater
- PE Bags

Laboratory supplies

- Technical balance
- Analytical balance
- Microwave digestion system equipped with Teflon vessels - Milestone Ethos One - Milestone
- Freeze-dryer (Recuperare specifiche)

Analytical eqiopement

- ICP-MS - Agilent 7500 ORS – Agilent Technologies Inc. Santa Clara, California

4.2 Samples

To develop the analytical method were used samples bought at the supermarket. The samples examined came from four different wheel of Asiago cheese. The cheeses list in table 4.1, differentiate for aging time.

Table 4.1: Description and labels of Asiago cheeses samples used for the method developement.

Label	Asiago	Type	Aging time	Paste description
FE	Fostered	Extra mature	15 months	Straw yellow, hard consistency. Sparse holes.
FM	Fostered	Medium seasoned	6 months	Pale yellow soft but not elastic consistency. Sparse holes of small and medium size.
PZ	Pressed	Fresh	20 days	White-pale yellow, irregular holes. Soft and elastic consistency.
PT	Pressed	Fresh	20-60 days	White-pale yellow, irregular holes. Soft and elastic consistency

The samples was reduced in small pieces, or scratched, where possible, in order to increase the contact surface, and support the reaction between reagents and samples. After crusts and the outer part removal from cheeses slices, extra mature Asiago was sampled using a plastic grater, and the softer Asiago cheeses were sampled taking small pieces with the aim of a PTFE spatula. The cheese powder and the small pieces were collected on PE bag, and then divided in two parts. An aliquot was stored in PE tubes, at $-20\,^{\circ}\mathrm{C}$, until digested, the second aliquot has been freeze-dried, and then stored in PE tubes, at room temperature.

4.2.1 Sample preparation

Samples preparation, for ICP-MS analysis, was carried out with the aim of a microwave digestion system Milestone ethos One. For each digestion run, the rotor was charged with

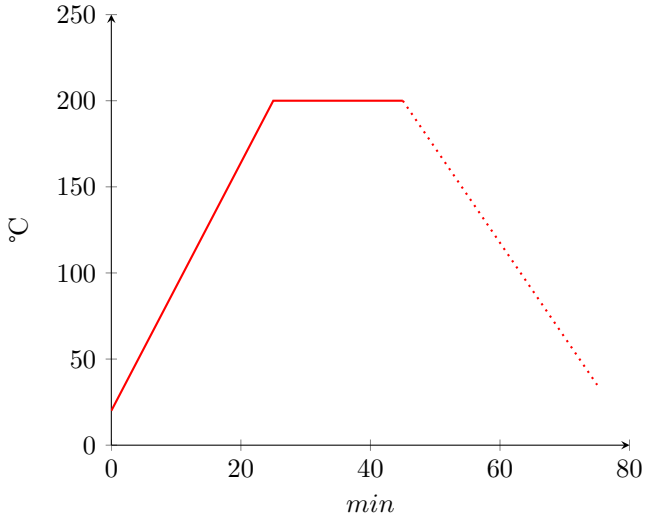
- blanks duplicate;
- SRM
- samples replicate

About 1 g of sample or RM was weighed inside the Teflon vessel and a mixture of 10 mL of HNO_3 and 5 mL of H_2O_2 were added as digestion reagents. Adding hydrogen peroxide caused a light fizz, due to the CO_2 formation, because of the organic matter breaking down. Blanks were obtained only with reagents in the vessels. To verify if a small enhancing in reagents volume could modify digestion performance, non freeze-dried samples were digested also with 12 mL of HNO_3 and 6 mL of H_2O_2.

Table 4.2: Summarised digestion methods.

Method	Sample	HNO_3	H_2O_2
A	As it is	10 mL	5 mL
B	As it is	12 mL	6 mL
L	Freeze-dried	10 mL	5 mL

The samples were then digested with a microwave cycle of 45 minutes, at a 200 °C. At the end, twenty minutes of vent was made to cool down the temperature.



	time (min)	Temperature (°C)
Heating	25	200
Manteinance	20	200
Venting	20	T amb

After microwave digestion, the bombs were removed from the oven, and let the samples cool to environment temperature before opening the vessels and transfer the obtained solutions to 50 mL polyetilen tubes. The solutions obtained were yellowish but limpid. The ones obtained from freeze dried samples was lighter-yellowish than the ones obtained from not treated samples. After every digestion run, the vessels and the shield were rinsed with milli-Q water, and a cleaning microwave cycle was performed, in order lo lower as much as possible the contaminations.

The samples was stored at −20 °C, and the opportunely diluted before ICP-MS analysis.

4.3 ICP-MS analysis

Measurements were carried out with an inductively coupled plasma quadrupole mass spectrometer (ICP-MS) (Agilent 7500 ORS – Agilent Technologies Inc. Santa Clara, California) equipped with an octapole reaction system (ORS) and autosampler. The instrument was settled with a V-groove nebulizer, a Peltier-cooled quartz spray chamber, and a quartz torch with a quartz injector tube.

The instrumental performance was monitored before starting the analysis session, acquiring a multi element tuning solution, containing about $1\,\mathrm{ng\,mL^{-1}}$ of $7Li$, $89Y$, $205Tl$ and $140Ce$. The instrumental performance optimization was carried out adjusting torch alignment, nebulizer gas flow rate, RF power and lens voltages. The instrument has been settled up to obtain oxide rate lower than 5% and doubly charged ions rate lower than 4%, and to enhance the sensitivity for monitored masses. The instrument was equipped also with a T valve for the injection of internal standard for the injection of internal standard solution (platinum and rhodium $1\,\mathrm{ng\,mL^{-1}}$) during analysis, in order to correct the temporal variations (drift or noise effects) in signal intensity. Pt and Rh were chosen as standard elements because they were supposed not to be present in the samples, and they are similar to the analyte for mass and ionization potential.

To lower as much as possible interferences, some masses were acquired with reaction cell, using He as reaction gas.

4.3.1 Calibration

Quantitative analysis was performed via external calibration, using ICUS -1675 as multi-element standard. Standard solution was gravimetrically diluted in order to obtain a $0.5\,\mathrm{ng\,g^{-1}}$ to $500\,\mathrm{ng\,g^{-1}}$ working range for each element. Calibration curves were calculated for each element, choosing the most proper regression fit (in most cases a weighted linear regression[1]). Linearity of calibration curve was good for the most of the monitored elemens(To evaluate calibration accuracy, some control standard were analysed during the analysis session whereas to evaluate method accuracy, RM samples were acid digested and analysed.

4.4 Data Analysis

Analytical data were exported as quantitative values ($\mathrm{ng\,g^{-1}}$), result of the average of 3 acquisitions. The analyte concentrations were calculated by instrument softwareon the basis of calibration curves, after signal correction by internal standard intenity, Concentration data were manually blank corrected and the samples analyte composition were calculated taking into account dilution steps and sample weight.

[1]The square reciprocal standard concentration of the corresponding standard is used as weighting factor for each point, to guarantee a better fit, for the lower concentration points.

Table 4.3: Calculated values of LoD and LoQ [ng g^{-1}].

Eelement	Mass	*LOD*		*LOQ*		Eelement	Mass	*LOD*		*LOQ*	
		$A+L$	B	$A+L$	B			$A+L$	B	$A+L$	B
Li	7	0.006	0.026	0.019	0.086	Ga	69	0.008	0.001	0.026	0.004
Be	9	0.006	0.003	0.021	0.009	As	75	0.015	*nd*	0.048	*nd*
$Al*$	27	0.673	0.685	2.243	2.283	$As*$	75	0.015	0.012	0.049	0.039
Ti	47	*nd*	*nd*	*nd*	*nd*	$Se*$	77	0.083	*nd*	0.278	*nd*
$V*$	51	0.011	0.003	0.035	0.009	$Se*$	78	0.730	0.167	2.433	0.557
$Cr*$	52	*nd*	*nd*	*nd*	*nd*	$Se*$	82	0.135	*nd*	0.451	*nd*
$Cr*$	53	*nd*	*nd*	*nd*	*nd*	Rb	85	0.008	*nd*	0.028	*nd*
Mn	55	0.006	0.012	0.019	0.041	Sr	88	0.013	0.001	0.043	0.004
$Mn*$	55	0.013	0.015	0.042	0.051	Y	89	0.008	0.003	0.027	0.011
$Fe*$	56	0.712	*nd*	2.373	*nd*	Mo	95	0.026	*nd*	0.086	*nd*
$Fe*$	57	0.842	1.554	2.806	5.181	Ag	107	0.010	*nd*	0.033	*nd*
Co	59	0.008	0.023	0.026	0.077	Cd	111	0.008	0.002	0.026	0.006
$Co*$	59	0.014	0.033	0.046	0.111	Sb	121	0.008	0.003	0.028	0.010
Ni	60	0.034	0.040	0.113	0.132	Ba	137	0.314	*nd*	1.046	*nd*
$Ni*$	60	0.070	0.064	0.233	0.213	Pb	204	0.027	0.006	0.089	0.021
Cu	63	0.026	0.010	0.088	0.032	Tl	205	0.013	0.003	0.042	0.010
$Cu*$	63	0.033	0.023	0.111	0.076	Pb	206	0.073	0.045	0.244	0.150
Cu	65	1.584	*nd*	5.281	*nd*	Pb	207	0.014	0.001	0.046	0.005
$Cu*$	65	1.084	*nd*	3.613	*nd*	Pb	208	0.028	0.044	0.093	0.147
Zn	66	0.060	*nd*	0.200	*nd*	Bi	209	0.015	*nd*	0.050	*nd*
$Zn*$	66	0.256	0.178	0.853	0.594						
Zn	68	0.068	*nd*	0.228	*nd*						
$Zn*$	68	0.068	*nd*	0.226	*nd*						

4.4.1 Limits of detection

Limit of detection is defined as the lowest quantity of a substance that can be detected with reasonable certainty for a given analytical procedure. Ddetection limits were calculated as:

$$LOD = 3 \cdot \sigma_b \tag{4.1}$$

where σ_b is the the standard deviation given by average values of the digestion blank measurements. Using digestion blanks for calculation includes in LoD background variation given by instrumental analysis and the background due to sample preparation procedures. The values above detection limits were excluded from data elaboration and in data handling are marked as $< LoD$.

Quantification limits are calculated as:

$$LoQ = 10 \cdot \sigma_b \tag{4.2}$$

$LoDs$ and $LoQs$ values, calculated for the differen methods are summarized in table 2.

4.4.2 Recovery

Recovery is a measure of the trueness of a measurement procedure[**pro**]. The analysys of a reference material the comparison of results with certified values can give an evaluation of method bias. To evaluate methods recovery Whole milk powder (SRM - 8435) was used as reference material.
The Reference material was acid digested following the procedure used for samples. Recovery was calculated comparing values obtained by instrumental analysis C_{Calc}, and the values reported in the SRM certificate of analysis C_{SRM}:

$$R = \frac{C_{Calc}}{C_{SRM}}\% \tag{4.3}$$

Recovery evaluation led to the exclusion of several elements from statistical analysis. The values calculated from SRM analysis showed a very high variability between a digestion run and another. The elements Table xxx reported in appendix summarize recovery calculated for the all isotopes, and elements for each method. The elements that revealed good recovery and good repeatability were selected to perform the further data evaluation(table4.4)

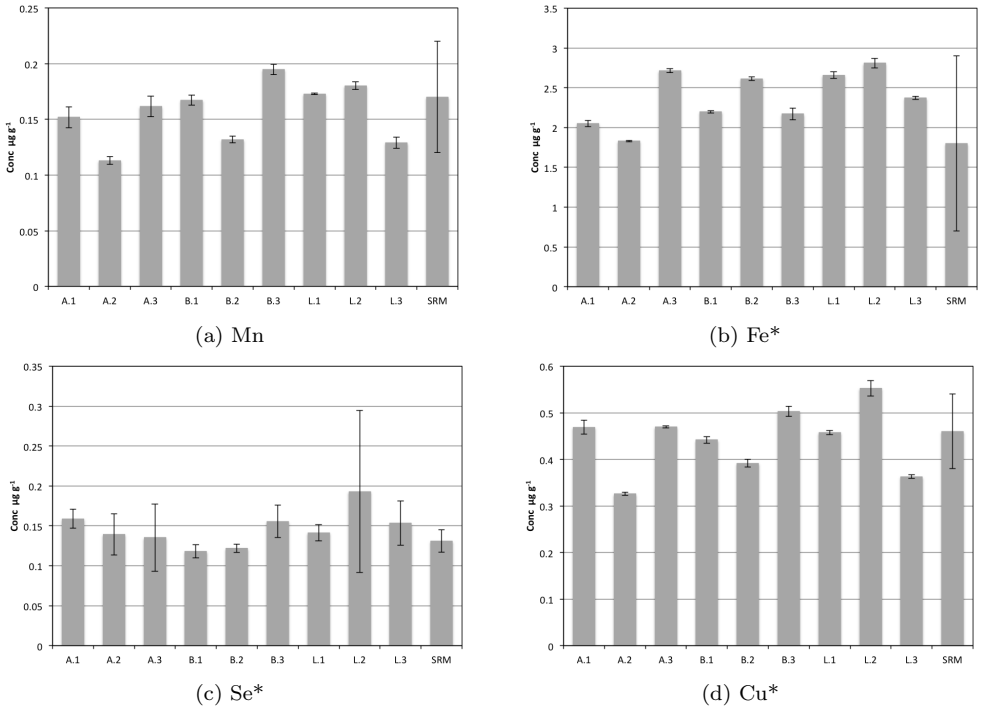
Between the selected elements the recovery values ranged from 60% and 164% with the uncertainty varying from $0,03\%$ to 10%, with the exception of selenium, which had in a couple of cases an RSD higher than 50%. Despite that Se was included in data analysis because the recovery calculated was always quite good, compared to the other elements. The recovery calculated was not always optimal but the values fell into the SRM concentration range plus or minus its uncertainty.(Figure 4.1).

Table 4.4: Recovery of selected elements reported for each digestion run. % values.

Eelement	Mass	*A*			*B*			*L*			SRM
		1	2	3	1	2	3	1	2	3	
Mn	55	89.3	66.5	95.0	98.4	77.5	114.6	76.0	106.0	101.7	100 ± 29.4
$Fe*$	56	113.9	101.7	150.8	122.1	145.2	120.5	131.7	156.1	147.7	100 ± 61.1
$Cu*$	65	102.0	70.8	102.1	96.0	85.1	109.3	78.8	120.1	99.5	100 ± 17.4
$Zn*$	68	97.9	75.2	100.0	103.7	84.4	110.1	89.9	109.2	100.2	100 ± 11.1
$Se*$	77	121.3	106.4	103.2	90.2	92.9	118.7	117.0	147.4	108.0	100 ± 10.7
Sr	88	131.3	79.9	137.0	112.1	117.4	163.5	90.4	120.3	148.8	100 ± 11.5
Mo	95	95.7	79.7	103.1	101.4	94.6	113.0	80.3	112.3	102.8	100 ± 44.8
Pb	204	117.0	80.2	94.0	91.1	86.7	157.4	59.5	92.1	143.3	100 ± 45.5

4.4.3 Repeatability

The method repeatability was evaluated digesting and analysing replicate of the same sample. Commonly the replicate was quite in agreement between each other. Data evaluation pointed out that freeze-dried samples concentrations were less variable within the replicates. This is particularly undeniable observing aged Asiago results as in figure 4.2.



(a) Mn (b) Fe*

(c) Se* (d) Cu*

Figure 4.1: Comparison of calculated concentration and cerified concentration.

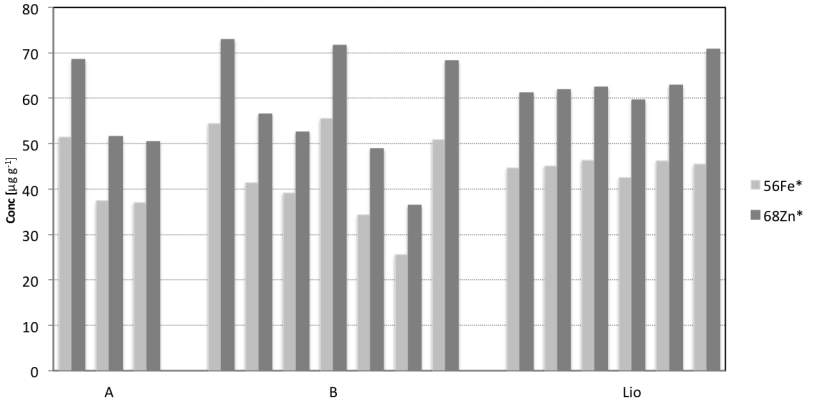


Figure 4.2: Extra mature Fe and Zn concentration [$\mu g\, g^{-1}$]. Comparison of replicates .

Table 4.5: Pressed Asiago Zogi and Tisato average concentration [$\mu g\, g^{-1}$] and RSD

Eelement	Mass	*Zogi*						*Tisato*					
		A		*B*		*L*		*A*		*B*		*L*	
		Conc	*RSD*	*Conc*	*RSD*	*Conc*	*RSD*	*Conc*	*RSD*	*Conc*	*RSD*	*Conc*	*RSD*
Mn	55	0.172	12%	0.229	19%	0.215	12%	0.124	25%	0.174	11%	0.161	12%
$Fe*$	56	30.3	11%	35.3	15%	34.7	13%	17.2	52%	26.0	7%	25.3	8%
$Cu*$	65	0.462	28%	0.463	18%	0.455	15%	0.235	52%	0.347	14%	0.334	13%
$Zn*$	68	37.6	11%	44.2	15%	43.4	12%	26.5	12%	33.0	7%	32.4	7%
$Se*$	77	0.139	15%	0.145	11%	0.143	11%	0.080	25%	0.103	12%	0.098	19%
Sr	88	4.62	13%	3.98	18%	3.92	16%	2.85	33%	3.85	10%	3.72	11%
Mo	95	0.092	13%	0.080	26%	0.078	25%	0.062	54%	0.111	63%	0.118	48%

Table 4.6: Fostered medium seasoned and extra mature Asiago average concentration [$\mu g\, g^{-1}$] and RSD

Eelement	Mass	$Medium - seasoned$						$Extra - mature$					
		A		*B*		*L*		*A*		*B*		*L*	
		Conc	*RSD*	*Conc*	*RSD*	*Conc*	*RSD*	*Conc*	*RSD*	*Conc*	*RSD*	*Conc*	*RSD*
Mn	55	0.242	8%	0.128	19%	0.230	5%	0.305	25%	0.325	29%	0.310	5%
$Fe*$	56	45.5	9%	25.3	17%	41.8	2%	42.0	20%	43.0	26%	45.0	3%
$Cu*$	65	10.2	9%	5.44	13%	9.65	2%	10.9	19%	11.4	25%	11.3	3%
$Zn*$	68	58.8	8%	32.3	14%	59.5	1%	56.9	18%	58.2	23%	63.2	6%
$Se*$	77	0.213	11%	0.102	23%	0.245	21%	0.147	16%	0.132	25%	0.189	13%
Sr	88	3.14	10%	1.72	17%	2.90	2%	3.08	22%	3.25	27%	3.27	8%
Mo	95	0.190	8%	0.092	21%	0.172	4%	0.242	23%	0.241	31%	0.269	6%

4.4.4 Samples elemental evaluation

The identified variation in elemental composition is related to the nature of the sample, but is also due to the preanalytical treatments. Analytical data summarized in table... revealed that pressed Asiago Tisato samples usually had the lower analyte concentration. Pressed Asiago Zogi samples had a slightly higher analyte concentration. A considering increasing in average concentration was observed mature samples and in the extra mature Asiago samples analysis.

This trend was observed for all the elements, except for strontium, which concentration was similar for all the cheeses samples. Those observations were always clear, independently from sample treatment. A higher residual standard deviation given from instrumental analysis has been registered for seasoned cheeses, especially in extra mature Asiago samples.

Manganese The isotope 55 of manganese was acquired both in normal mode and with the reaction cell. The acquisition in normal mode gave a good recovery with a better RSD. The concentration range registered within the batch samples varied from an average value of $0.124\,\mu g\,g^{-1}$ registered for pressed Asiago Tisato to an average value of $0.325\,\mu g\,g^{-1}$for extra mature Asiago, with good values of RSD ranging from 0.05% to 7%.

Iron Iron isotopes 56 and 57 were acquired only in reaction mode in order to reduce the interference due to the presence of polyatomic species like ArO^+, CaO^+. The better resolved isotope was 56Fe. The calculated concentration ranged from average values of about 20 $\mu g\,g^{-1}$ for pressed Asiago and about $40\,\mu g\,g^{-1}$ for seasoned Asiago. Instrumental RSD never exceeded 12%.

The different sample treatment lead to slightly difference in analyte concentration.

Copper The isotopes 63 and 65 of copper were acquired with and without reaction cell. The better recovery was calculated for 65Cu, in reaction mode.He collision inreaction cell lowered the polyatomic interferences, leading to a good data reading. The lighter isotope instead showed bad recovery for both acquisition modes. Copper concentration carried between $0.24\,\mu g\,g^{-1}$ in Tisato cheese samples and $11.4\,\mu g\,g^{-1}$ in extra mature Asiago samples. The RSD calculated on the average instrumental acquisition were included between 0.1% and 3%.

Zinc Zinc instrumental acquisition was performed with and without reaction cell, and the concentration monitored regarded the isotopes 66 and 68. As expected the better recovery and the lowest error was calculated for the isotope 68 acquired with the reaction cell. The lowest average concentration ($26.5\,\mu g\,g^{-1}$) was registered in Tisato pressed sample while the samples most rich in Zinc were the seasoned ones, with an average calculated concentration of around ($63\,\mu g\,g^{-1}$). The instrumental RSD for 68Zn, never exceeded 4%.

Selenium Selenium analysis was performed on masses 77, 78 and 82, using for all the reaction mode acquisition. As expected Se analysis was not a trivial matter because this non-metal is interfered from polyatomic species due to the presence of Ca, N and S in the organic matrix. The better recovery was calculated for 88Se, and its concentration in cheeses samples was quite homogeneous, raging around $0.15\,\mu g\,g^{-1}$ but the uncertainty related to the values is very high, especially for the seasoned samples.

Strontium The mass selected for Sr evaluation was the 88, that is low interfered, and can be quantified in normal mode. The concentration of strontium is similar in the samples, even if a slightly higher average concentration was calculated for fresh Asiago ($4.5\,\mu g\,g^{-1}$). The RSD ranged from 0.2% to the highest value of14%, related to the analysis of a extra mature Asiago.

Molybdenum As Sr, Mo was acquired only in normal mode. The concentration values ranged from $0.1\,\mu g\ g^{-1}$ of pressed cheeses to $0.25\,\mu g\,g^{-1}$ of extra mature Asiago. Residual standard deviation ranged from 0.3% to 10%, with the exception of few outliers.

Lead The lead isotopes 204, 206, 207 and 208 were acquired in normal mode. RM analysis gave good results and the better recovery was calculated for 204Pb. Lead analysis in cheese samples did not reach satisfactory results. RSD was very high, and the presence or the absence in the samples could not be ensured because of the high variability in the results.

4.5 Statistical analysis

In order to evaluate whether the different Asiago samples could be discriminated on the basis of their elemental composition, a chemometrical approach was used. Cluster analysis and principal component analysis was performed to explore the multivariate space, that consisted in 58 cases and 7 variables. To perform statistical analysis specific software were used, as Sratistica and R.

Cluster analysis The principal aim of cluster analysis is to find out groups within a dataset. A hierarchical cluster analysis was applied to the Asiago data set, to understand whether the samples could be grouped on the basis of their elemental composition and if the preanalytical procedures affect the classification.

Tree diagram in figure 4.3 is the result of the performed cluster analysis, where the correlation between variables was calculated on Pearson, and the linkage used was Ward's method.
Observing the tree diagram for cases can be clearly distinguished two biggest blocks, highly separated. The group on the left is composed of fostered asiago samples, and the other one is composed of pressed Asiago samples. This means that the two variety of Asiago cheese can be distinguish on the basis of their composition. Looking inside those macro groups, the situation is less defined, especially for pressed samples.

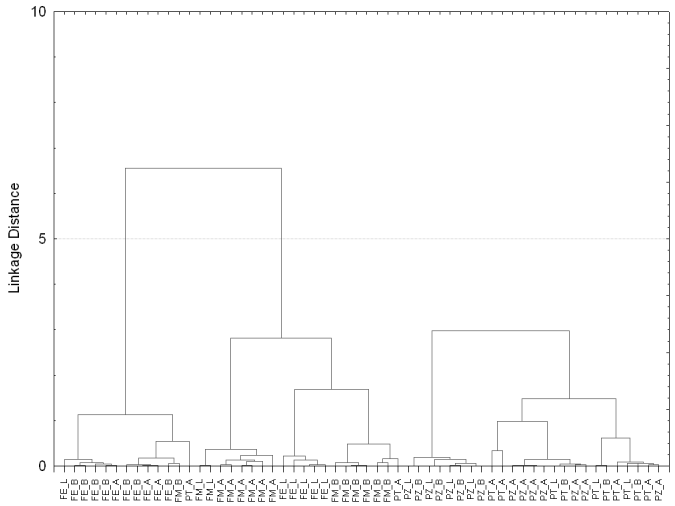


Figure 4.3: Tree diagram for 58 cases. Pearson correlation, Ward's linkage method.

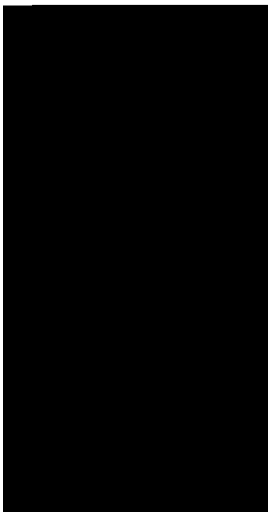
This can be lead back to the preanalytical sample preparation that, as seen before, in method A an B showed some repeatability lack, that can induce in wrong classification. Concerning pressed samples, the hurdle in separation can be due to the high similarity in sample seasoning. To overcome variability due to preanalytical, and to understand how much this step affected the final results, cluster analysis was performed on tree subset of the dataset, dividing data on the basis of the sample preparation methods.

As shown in figure 4.4, method A and method B sample preparation did not lead to a complete and correct separation, even if the analysis roughed in samples classes. Instead the freeze-dried samples cluster analysis perfectly divided the samples in 4 subgroups.

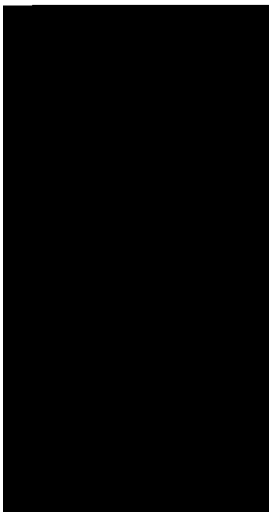
Tree diagram of variables (Figure 4.5) revealed high correlation especially for Zn, Fe and Mn, while Sr and Se result less correlated .This trend is the same both 58 cases diagram than subgroup diagrams.

Principal component analysis Principal component analysis (capitolo....)is a projection technique used to explore multivariate data space. This tool is useful because can highlight relationship among object and variables (cit chem in food chem). PCA was performed on data matrix composed of 58 cases x 7 variables.

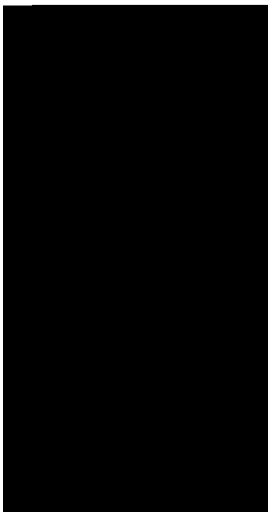
The loadings and scores plots explain 71.21% of the total variance in component 1 and 17.35% of the total variance in component 2 PCA score plot (figure 4.6) illustrates a clear separation pattern between pressed Asiago samples and Fostered Asiago samples. The corresponding loading plot(figure 4.7) describes the variables relation with sample separation. Elements selected for PCA analysis control the discrimination of pressed and fostered Asiago, but they only suggest a partial separation of mature to extra-mature samples. Also the two pressed cheeses cannot be clearly distinguee. Loading plot shows that Fe, Mn and Zn are highly correlated, as already



(a) Method A - tree diagram for 22 cases. Union linkage distance 15.



(b) Method B - tree diagram for 21 cases. Union linkage distance 12.



(c) Method L - tree diagram for 15 cases.Union linkage distance 10.

Figure 4.4: Tree diagrams. Pearson correlation, Ward's linkage method.

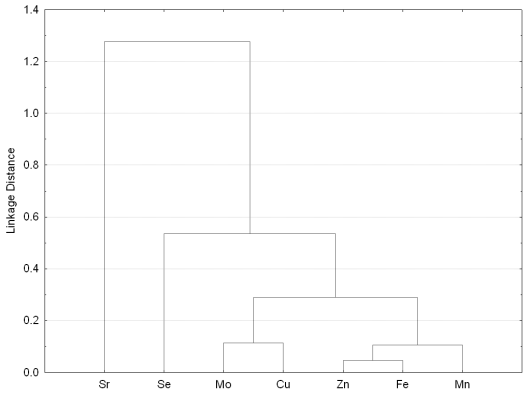


Figure 4.5: Tree diagram for variables. Pearson correlation, Ward's linkage method.

demonstrated in variable dendogram in figure 4.5, those elements totally grave on the first component. Instead Sr produce the separation in the 2nd component, and is totally uncorrelated to the other elements.

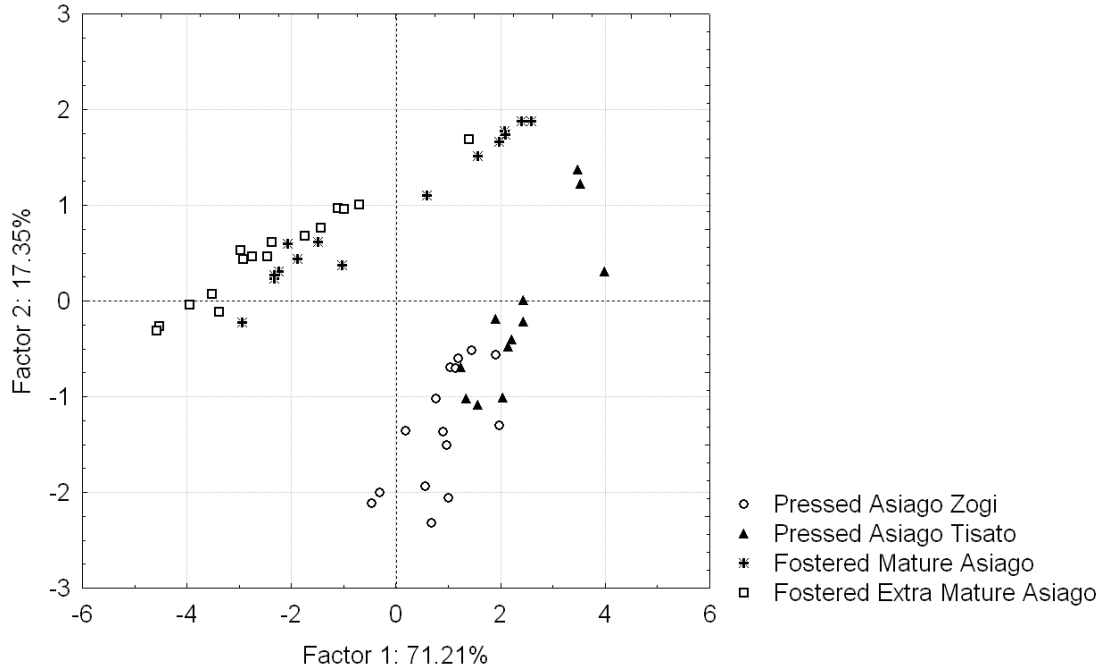


Figure 4.6: Projection of the cases on the factorplane (1x2)

Even if the data were too few to make proving evaluations, a PCA was performed on freeze-dried samples data, using a matrix of cases x 7 variables . The samples displayed in score plot in figure 4.8 resulted clearly separated.

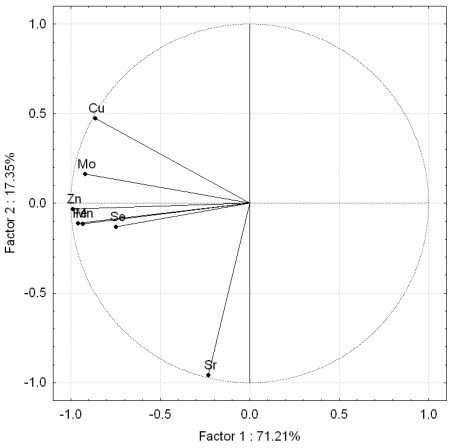


Figure 4.7: Projection of the cases on the factorplane (1x2)

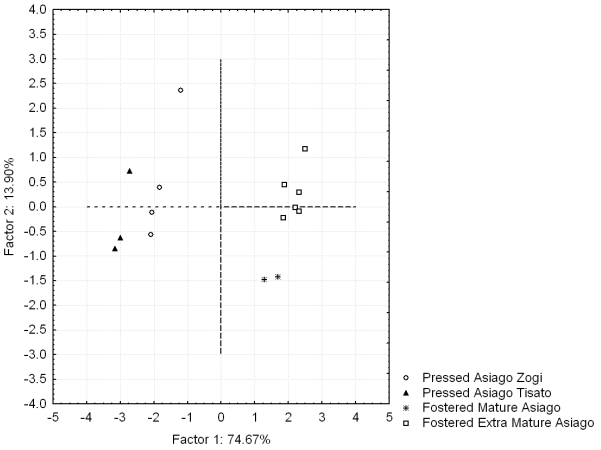


Figure 4.8: Projection of the cases on the factorplane (1x2)

4.6 Conclusion

This work was developed at the beginning of my PhD period, and it was thought as a preliminary study, a starting point for a wider authentication study on Asiago cheese. Due to some events the research was left in standby.

Even if the work is incomplete, some consideration can be done on the analytical results. Sample preanalytical treatment turned out to be a fundamental step to obtain precise results. Cheese freeze-drying was proved to be a smart preanalytical choice. Good results in repeatability revealed that treated samples were more homogeneous, leading to a better analytical data. Beside that freeze-dried samples can be easily stored at room temperature, which is not pokey, because cheese is a perishable matrix. No significant improvement was noticed in recovery using a higher volume of reagents but to improove digestion step.

Multivariate statistical analysis applied on multi-element fingerprint is a powerful tool for food classification. Improvements in the analytical method in order to obtain a higher number of variables, could lead to a better discrimination between samples. Even if the method was not completely developed, Asiago cheese samples could be discerned, at least on aging time. Seasoned samples showed a more characteristic and "intense" fingerprint. Pressed samples has an elemental composition that allowed distinguishing them from fostered Asiago, but the data collected did not permit a significant differentiation between the two cheeses. This can be due to the limits of the developed technique. Is also true that pressed Asiago is a fresh dairy product that, if is produced with milk having the same origin, do not have the time (given by long time seasoning) to develop singular characteristics.

To improve the research, method development should be extended to a widest number of analyte in order to obtain better discriminant parameters.

Analysis of isotope ratios of bio-elements like carbon, nitrogen, oxygenhidrogen and sulphur can be a very powerful instrument for origin identification and authenticity investigation that can be complementary to elements composition study. Morehover, Sr isotope ratio canf furnish additional information, highly correlated to the geologichal background.

Part III

Barley

Chapter 5

Parte generale

The most consistent part of my PhD research regards analytical method development for traceability study of barley and malt.

Barley with wheat, rye, rice, millet and oats, is a major cereal grain, and it is amongst the most important food for mankind and also for breeding animals. Barley is a high efficiency cereal, and due to its strength and versatility gives productive results even in difficult conditions, leading to high profits. Due to its content of β-glucans[1] , barley has an increasing importance in the field of health food. Barley's flour is used in production of bread, pasta, and cookies with healthy properties.

Barley is widely used in malt production. Brewing sector is an increasingly important part of the Italian agrifood industry. Those are the reasons prompting Italian agriculture to find new formulas to characterize the quality of the barley grown in it's own territory. Scientific research yields to genetic improvement of the species, generating seeds with an higher functional molecules content, for health scope, or with a lower protein content, for malting and brewing purpose. In this respect, the CRA[2] research centre of Fiorenzuola d' Arda (PC) performs various research activities on barley and functional foods, with a particular attention to the study of genetic variability, nutritional and biochemical characteristics of the products and their technological implications. My PhD research work was developed with C.R.A.– Genomics Research Centre, in Fiorenzuola d'Arda (PC) support. They furnished barley, malt, malting process threshes, and soils samples for the provenance study.

[1] Linear polysaccharides that has salutary effects on controlling glucose in the blood and on maintenance of normal blood cholesterol values.

[2] The Agricultural Research Council (CRA) is a National Research Organization that operates under the supervision of the Ministry of Agriculture, with general scientific competence within the fields of agriculture, agroindustry, food, fishery and forestry.

5.1 Barley

Barley is an herbaceous annual plant, and the one commonly cultivated belongs to the species $Hordeumvulgare$.
It can be classified according to the number of rows of grains of the ear. If only the central spikelet of each node of the rachis is fertile and the two sides are sterile, the ear carries only two ranks and has a strongly flattened shape: these are the two raw barley ($Hordeumvulgaredistichon$). In the six-raw barley ($Hordeumvulgarehexastichum$) instead are fertile the three spikelet on each node of the rachis. Two-row barley has a lower protein content than six-row barley, thus more fermentable sugar content.
Barley has a high adaptability to marginal environments and its short life cycle allows to be grown almost to the Arctic Circle where it is the only cereal that gets ripen in the short summers. Barley is also preferred to wheat in dry environments due to barley's relatively low water consumptions and good tolerance to high temperature, for this reason barley is the dominant cereal in semi-arid areas of the Middle East and North Africa. In Tibet, Nepal, Ethiopia, and the Andes, farmers cultivate barley on the mountain slopes at elevations higher than other cereals. In areas with little irrigation in the dry regions of North Africa, the Middle East, Afghanistan, Pakistan, Eritrea, and the Yemen, barley is often the only suitable cereal.
Presumably barley is the first farmed cereal, it was first domesticated in the Near East/Fertile Crescent region, and until XV sec it was between the most diffuse cereals for breading. Nowadays the main barley growing countries in the world are Russia, Canada, Germany, France, Ukraine, Spain, Turkey, UK, Australia, USA, and Denmark.

5.2 Malt

The forced to germination grains of cereals (barley, wheat, rye, oats, rice) are called malt. When the malted cereal is not specified, the term is implied referred to barley malt. Maltation takes place in differen steps, and the main are steeping, germination, and kilning. Soaking the kernels 36-48 hours hydrates the raw cereal to help along germination. During germination, while bud sprouting, the starch molecules become soluble starch, simple sugars and a large part of enzymes that play a key role in mashing are produced. The longer last germination, the more highly modified the malt. The germinated barley, before being dried is called "green malt". Green malt is then passed to the kilning step, necessary to stop germination and to dry the malt as much as possible. The process is performed in tree steps, at low temperature - around 32°C the first and 50°C the second - to preserve malts' enzymes. The final toasting step, (80°-100°C) influences the malt taste, conferring specific organoleptic characteristics to the product.

Barley malts are used mainly for brewing, distillates productions, and food production. Moreover, wastes produced by malting process are used for feeding animals.

Chapter 6

Experimental

Sample preparation, standard dilution and all the laboratory work has been performed in a class ????? clean room, to reduce contaminations risk, due to the working environment.

6.1 Materials and Methods

Reagents and standards - Multi-element analysis

- Nitrico tecnico da distillazione
- Nitric acid 65% Suprapur - Merck KGaA, Darmstadt, Germany
- Nitric Acid UpA 67-69% - ROMIL Ltd, Cambridge, GB
- Hydrogen peroxide Suprapur - Merck KGaA, Darmstadt, Germany
- Hydrogen Peroxide 30-32% - UpA ROMIL Ltd, Cambridge, GB
- High-purity water 18.2 MΩ - Elga LabWater system, Veolia Water VWS, UK
- Ammoniaca UPA
- Multi elemental standard ICUS1675-
- IMS-102 ICP/MS Calibration standard #2 - ULTRA Scientific North Kingstown, USA
- IMS-101
- Standard Reference Material 1567 *Wheat Flour*
- Pt standard
- Rh standard
-

Laboratory ware - Multi-element analysis

- Watch Glasses Polypropylene
- 50 mL PP centrifuge tubes –
- 15 mL PP centrifuge tubes –
- 50 mL PP digestion tubes SCP Science, Quebec - Canada
- PTFE Spatulas
- Ceramic mortar
- PE Bags
- Bottiglie nalghene
- Filtri 0.45
- Minisart® SRP15 Filters - Sartorius - Goettingen, Germany
- Setaccio 2mm

Laboratory supplies - Multi-element analysis

- Technical balance
- Analytical balance
- Kjeldahl Digestion Units - DK Series - Monza e Brianza - Italy
- Microwave digestion system equipped with Teflon vessels - Milestone Ethos One - Milestone
- Ball Mill
- DigiPrep - Graphite heating block
- DISTILLATORE
- STUFA ´(per suoli)
- Setacciatore

Analytical eqipement - Multi-element analysis

- ICP-MS - Agilent 7500 ORS – Agilent Technologies Inc. Santa Clara, California AUTO-CAMPIONATORE

Samples

6.2 Method developement

6.2.1 Sample preparation

Samples At the very beginning of this study, before working seeds samples furnished by CRA , the analytical method for elemental analysis was developed using commercial oat flakes and commercial pearl barley. Also commercial malt was used, in particular, Maris Otter malt and Vienna Malt. Maris Otter malt is a malt with low protein content. It's produced from the homonymous English barley variety, dried at very low temperature It is used as principal ingredient for English style beers. Vienna malt is kiln-dried barley malt, traditionally makes up to 100% of the grist of Vienna Lager and the bulk of the related Märzen style. It's used for brewing amber beers.

To homogenise samples and facilitate the matrix dissolution, cereals were grinded before acid digestion. At the beginning a ceramic mortar was used. Then a mechanical ball mill was used to homogenize samples. Cereals seeds samples were grinded in teflon vessels for 20 minutes, with a frequency of 25 1/s.

6.2.2 Open vessel digestion

Sample preparation To homogenise samples and facilitate the matrix dissolution, cereals samples were grinded before sample acid digestion. At the beginning a ceramic mortar was used. Then a mechanical ball mill, equipped with Teflon vessels, was used to homogenize samples.

Open vessel digestion The first approach to sample preparation for the determination of metals in cereals samples was based on open vessel wet digestion. Samples preparation was mostly performed in 50 mL polypropylene tubes, with a graphite block DigiPREP digestor. A test was also performed with a Kjeldahl Digestion Units, with 250 mL Pyrex tubes. Sample and SRM were weighted on weighing boats and then transferred in to the tubes, where nitric acid and hydrogen peroxide were added. Tubes were than placed in the heating block, and the temperature was enhanced to digest samples according to the processing procedure. Many tests were carried out in order to evaluate the better mineralization procedure. Each digestion set was composed of replicate blanks, standard reference material and replicate samples. Before performing acid digestion polypropylene and Pyrex tubes, caps, and clock watches were soaked overnight in a 2% nitric acid bath, and then rinsed with milli-Q water. Pyrex tubes were also subjected to a cleaning digestion run The tubes were filled only with reagents, and heated with the digestion block up to $200C$ for half an hour. After heating, tubes were rinsed again with ultrapure-water.

Procedure A About 0.5g of oat sample was placed into the PP digestion tube, and then 6mL of HNO3 and 4mL of H2O2 were added. The samples were digested at 100°C for 2 hours, and the vessels were closed with their caps. Before starting acid digestion, the samples were let 12 hours at environmental temperature.

Procedure B and B' About 0.5g of sample was weighted and transferred into the PP digestion tubes. A mixture of 4mL HNO3 and 2 mL H2O2 was added and the tubes was placed in the digestion system. In the first digestion step the temperature was kept 30 min at 50°C, then for other 30 min at 70°C and then 1 hour at 100°C. After cooling to environmental temperature the solution, mL4 HNO3 and 2mL H2O2 were added the samples were digested again, 30min at 80°C and then 1 hour at 80°C. In procedure B tubes were closed with caps during digestion process and in procedure B' PP watch glasses were used.

Procedure C - C' and D – D' A mixture of 6mL of HNO3 and 2mL of H2O2 was added to the samples inside the digestion tubes. The sample amount was about 0.5g in procedure C and C', instead 0,25g was weighted in D and D'. The mixtures were heated 30min at 100°C and after cooling down to environmental temperature, 2mL oh H2O2 were added to the solution, and heated again at 100°C for 1 hour. Vessels C and D were closed with their PP caps, while C' and D' PP watch glasses were used.

Procedure E An amount of 0,25 g of sample were dissolved in 5mL of HNO3, heated in polypropylene tubes for 15 min at 100°C. After cooling, other 2,5mL of nitric acid were added, and the samples were heated again at 100°C for 25 minutes. After digestion 1,5mL of hydrogen peroxide was added in the tubes, turning to yellow the solutions. Once sparkling ended, 1 mL more was added to the tubes, and the solutions were placed again in the heat-bock, for a 40 minutes digestion, at 100°C.

Procedure F The last open vessel digestion test was performed with a Kjeldahl Digestion Units, using 250mL Pyrex test tubes. About 0,5g of samples were weighted in weighting boats, and placed into the tubes with HNO3 (6mL) and H2O2 (4mL). The block temperature was enhanced with a heating ramp that kept 50°C, for 30minutes 100°C for 30 minutes, 150°C for 30 minutes and 200°C for 30minutes. After cooling, the solutions were transferred to polypropylene tubes.

After each different digestion procedure, the solutions were left to cool downand then diluted up to 50 mL with ultrapure-water. Samples were stored at -20°C prior to analysis. Each digestion set was composed of replicate blanks, replicate standard reference material and replicate samples.

6.2.3 Microwave digestion

Several tests were also performed using a MW digestor system in order to evaluate the most accurate and precise preanalytical procedure. Tests were performed using a digestor system Milestone Ethos One, equipped with a rotor composed of ten high-pressure digestion vessels. Milled samples were weighted directly in the microwave vessels, and then the reagents were added. Each digestion run was composed of replicate procedural blanks Replicate samples Standard reference material The obtained solutions were transferred in PP tubes, diluted up to 50 mL and stored at -20°C until analysis. After every samples digestion run, microwave vessels were cleaned by rinsing them with UP water prior and after a MW cleaning run, using a mixture of HNO_3 and H_2O_2.

In **procedure MW-1**,**MW-2** and **MW-3** about 0.5 g of milled oat flakes was transferred in to a Teflon bomb, and mixture of HNO_3 supra-pure and H_2O_2 Supra-pure was added prior to perform the digestion using the MW digestion program n°1 6.1 .The three MW digestion procedures were performed changing the reagents ratio. In procedure MW-1 6 mLof HNO_3 and4 mLof H_2O_2. In MW-2 was used the same amount 5 mL for the two reagents. In procedure MW- 3 instead 7 mLof HNO_3 and3 mLof H_2O_2 were added in to the digestion vessel. Solutions obtained were transferred into PP tubes and diluted with UP-water up to 50 mL.

About 0.5 g was weighted inside the digestion vessel in ***procedure MW-4***. An amount of 6 mLof HNO_3 and4 mLof H_2O_2, but instead of supra-pure, UpA nitric acid was used. The samples were digested with digestion program n°1. Sample were then transferred to PP tubes, and diluted up to 50 mL before being stored. ***procedure MW-5*** and ***MW-6*** were performed as procedure MW-4, but changing microwave digestion ramp to program n°2 6.1. After digestion samples were transferred to PP tubes. In procedure MW-6 samples were transferred in tubes previously acid washed, unlike in MW-5 (and all the others procedures), tubes were used without being washed. In ***procedure MW-7*** 0.5 g of sample were digested with 6 mLof HNO_3 and4 mLof H_2O_2, testing double subboiled nitric acid. In procedure MW-5, MW-6 and MW-7 were acid digested oat flakes and barley samples.

Some tests were also performed on malt samples. In ***procedure MW-8*** 0.5 g of malt was digested with microwave program n°2 6.1, using 6 mLof HNO_3 double subboiled nitric acid and4 mLof UpA H_2O_2. Two slower temperature ramp, digestion program n°3 and n°4, were tested in ***procedure MW-9*** and ***MW-10*** , keeping constant the other parameters.

In the last digestion digestion set, ***procedure MW-11***, about SI0.5g of sample was transferred into digestion vessel, and dissolved wit 6 mLof HNO_3 double subboiled, 3 mLof UpA H_2O_2 end 4 mLof UP water. Samples were then digested with MW program n°1 6.1.

After digestion the obtained solutions were transferred in PP tubes, and stored at -20°C prior to analysis.

Table 6.1: Microwave oven digestion programs.

Step	1		2		3		4	
	$t$min	T°C	$t$min	T°C	$t$min	T°C	$t$min	T°C
Heating	10	200	5	100	10	100	10	80
Manteinance	15	200	7	100	10	100	10	80
Heating	–	–	10	200	10	200	10	100
Manteinance	–	–	15	200	15	200	10	100
Heating	–	–	–	–	–	–	10	200
Manteinance	–	–	–	–	–	–	15	200
Ventilation	20		20		20		20	
$ttotal(min)$	45		57		65		85	

Table 6.2: •

Test n°	Sample		Reagents			Microwave program	Note
	Peso	Type	Vol	Type	Grade		
1	0.25	Oat	6 mL 4 mL	HNO_3 H_2O_2	Supra Supra	1	
2	0.25	Oat	5 mL 5 mL	HNO_3 H_2O_2	Supra Supra	1	
3	0.25	Oat	7 mL 3 mL	HNO_3 H_2O_2	Supra Supra	1	
4	0.5	Oat	6 mL 4 mL	HNO_3 H_2O_2	UPA Supra	1	
5	0.5	Barley-Oat	6 mL 4 mL	HNO_3 H_2O_2	UPA Supra	2	
6	0.5	Oat	6 mL 4 mL	HNO_3 H_2O_2	UPA Supra	2	Decontaminated tubes
7	0.5	Barley-Oat	6 mL 4 mL	HNO_3 H_2O_2	UPA/Bidist Supra	2	
10	0.5	Malt	6 mL 4 mL	HNO_3 H_2O_2	Bidist UPA	2	
12	0.5	Malt	6 mL 4 mL	HNO_3 H_2O_2	Bidist UPA	3	
13	0.5	Malt	6 mL 4 mL	HNO_3 H_2O_2	Bidist UPA	4	
17	0.5	Malt	6 mL 3 mL 4 mL	HNO_3 H_2O_2 H_2O	Bidist UPA	1	

6.2.4 ICP-MS analysis

Samples analyses were performed in different days, along the first two years of research. Multi-elemental measurements were carried out with an inductively coupled plasma quadrupole mass spectrometer (ICP-MS) (Agilent 7500 ORS – Agilent Technologies Inc. Santa Clara, California), equipped with an octapole reaction system (ORS). The ion source was composed of a Peltier-cooled quartz spray chamber, and a quartz torch with a quartz injector tube. The instrumental performance was monitored before starting every analysis session, acquiring a multi element tuning solution, containing about $1\,\mathrm{ng\,g^{-1}}$ of 7Li, 89Y , 205Tl and 140Ce. The instrumental performance optimization was carried out adjusting torch alignment, nebulizer gas flow rate, RF power and lens voltages. An AUTOSAMPLER was used for solutions acquisition. In order to correct the temporal variations in signal intensity, a platinum and rhodium $10\,\mathrm{ng\,g^{-1}}$ standard solution was added on-line. To lower as much as possible isobaric interferences the reaction cell was used (Tab masse REACTION CELL), with He and H_2 as reaction gas.

Open vessel samples analysis

The instrument was settled with a V-groove nebulizer for the analysis of samples open vessels digested. LIST OF ANALYTES were acquired both in normal mode, and with the reaction cell, using He as collision gas. Quantitative analysis was performed via external calibration. Stock solution ICUS-1675 was gravimetrically diluted in order to obtain a calibration range varying $0.5\,\mathrm{ng\,g^{-1}}$ to $500\,\mathrm{ng\,g^{-1}}$

Microwave samples analysis

A concentric nebulizer was built for the analysis of microwaved digested samples. Elements were acquired in normal mode, and with reaction cell, using Helium or Hydrogen gasses. Quantitative analysis was performed via external calibration. Stock solutions IMS-102 and IMS-101 was gravimetrically diluted in order to obtain a suitable working range for each element. Calibration curves were calculated for each element, choosing the most proper regression fit.

6.3 Rare earth elements Analysis

Rare earth elements analysis of digested samples turned out to be quite tricky. Difficulties in REEs are related to the very low concentration of those analytes in food matrix. Furthermore in organic samples LREEs can form oxides species that create interferences on HREEs [[**Spalla2009**],[**Gabrielli2006**]]. REEs analysis of digested samples did not lead to any satisfactory results. Instrumental RSDs were very high, up to X% and quantification was possible occasionally only for Y, La and Ce . Analytes concentration were low, often the signal wad comparable to those of blanks. To improve REEs analysis, three digested samples were analysed with different instrumental settings, in order to find out satisfactory operative condition for their quantification. As

reported in Tomato et al, the ICPQMS was equipped with a high efficiency sample introduction system (ApexQ – Elemental Scientific, Omaha, NE, USA), in order to lower the solvent injection into plasma. Solutions were introduced both with direct suction and with autosampler trough peristaltic pump. Tests were also performed on not diluted samples, enhancing integration time for single elements, and numbers of acquisition per samples (Tab.6.3)

Table 6.3: Aquisition time [s]

Test	Aq.n°	Y	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
1	3	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.9	0.9	0.9	0.9	0.9	0.9	0.9
2	3	0.6	0.6	0.6	0.6	0.6	0.6	0.6	1.5	0.9	0.9	0.9	0.9	1.5	0.9	1.5
3	5	0.6	0.6	0.6	0.6	0.6	1.5	1.5	1.5	1.5	1.5	3	1.5	3	3	3

The instrumental sensitivity increased using an Apex as introduction system, leading to a signal enhancement up to 7 times, especially for LREE. Sample uptaking by peristaltic pump gave the better results in terms of cps but the signal resulted to be sensitive to the peristaltic pushes, creating a fluctuation in the signal. ApexQ, used with direct suction gave a slightly better signal compared to the first acquisition mode, but in term of automation of the procedure, resulted totally inconvenient because it was not possible to use it with the auto-sampler. The analysis of not diluted samples gave good results. Enhancing the acquisition time per element, and the number of acquisition per sample, produced an improvement to signal intensity (Fig. 6.1). Working with a solution with a high concentration (about 12%) of HNO_3 is usually not recommended, because it accelerates tubing connection and surfaces degradation and can lead to create isobaric molecular ion interferences with the analytes. (EPA method 6020 A) For this reason extra washing steps was introduced between samples, in order to clean up as much as possible residual from the previous samples. A fundamental step in REEs analysis is the instrumental tuning. For REESs analysis tuning step was performed in order lo lower as much as possible oxide formation. Furthermore enhancing time/number of acquisition, the instrumental RSD was quite restrained.

Data Analysis - Major and trace elements

Analytical data were exported as quantitative values $\mathrm{ng\,g^{-1}}$, result of the average of 3 acquisitions. Elements concentrations were calculated by instrument software on the basis of calibration curves, after signal correction by internal standard intensity. Concentration data were manually blank corrected and the samples composition were calculated taking into account dilution steps and sample weight.

Table 6.4: Signal intensity of REEs tests.

	Normal acquisition						APEX,peristaltic pump					
	*Samp*1		*Samp*2		*Samp*3		*Samp*1		*Samp*2		*Samp*3	
	CPS	*RSD%*	*CPS*	*RSD%*	*CPS*	*RSD%*	*CPS*	*RSD%*	*CPS*	*RSD%*	*CPS*	*RSD%*
La	604.62	5.79	720.86	6.66	770.58	0.48	3656.40	2.45	2298.41	3.54	3033.35	8.16
Ce	1214.58	2.68	1413.92	6.87	1455.57	2.76	6897.34	2.49	4016.56	4.97	6176.90	25.41
Pr	160.92	6.05	186.73	16.69	200.06	4.00	1171.92	3.46	701.43	6.00	929.79	10.81
Nd	107.35	8.24	133.57	8.64	146.70	4.94	855.11	3.56	531.62	5.75	639.36	8.13
Sm	29.31	10.46	34.96	3.93	37.49	13.05	158.25	5.92	106.63	4.29	123.86	5.23
Eu	107.57	10.66	116.00	10.82	104.57	10.79	643.90	12.54	358.17	13.12	469.51	15.27
Gd	29.98	8.88	40.19	20.49	31.35	20.98	187.57	5.64	120.35	8.44	145.98	12.21
Tb	27.95	9.37	27.05	10.72	30.83	6.64	159.04	5.39	99.32	9.25	126.93	19.47
Dy	31.28	2.20	36.38	14.49	36.43	6.60	224.42	14.09	155.17	8.43	151.16	12.57
Ho	31.90	13.07	32.17	5.52	26.99	9.08	176.06	3.98	115.52	5.31	136.46	25.54
Er	29.22	21.91	34.86	31.19	25.84	9.40	159.04	12.11	105.73	8.24	110.30	12.51
Tm	24.68	21.15	21.24	20.58	17.04	10.65	71.51	8.88	44.73	8.97	46.36	18.02
Yb	19.78	15.34	21.75	9.05	24.21	22.41	91.49	5.32	71.02	8.72	75.74	9.66
Lu	18.26	14.20	22.64	9.23	19.02	13.00	68.59	14.27	41.45	5.70	50.13	5.61

	APEX,direct suction						Not diluted sample, condition 1					
	*Samp*1		*Samp*2		*Samp*3		*Samp*1		*Samp*2		*Samp*3	
	CPS	*RSD%*	*CPS*	*RSD%*	*CPS*	*RSD%*	*CPS*	*RSD%*	*CPS*	*RSD%*	*CPS*	*RSD%*
La	1240.55	6.84	985.29	2.34	933.30	2.80	2450.51	10.05	1872.82	4.18	2102.87	4.83
Ce	2235.93	11.72	1817.73	13.36	1687.32	8.00	4631.15	7.76	3377.32	5.46	3666.54	7.46
Pr	322.38	11.77	268.55	12.98	253.23	6.74	597.97	1.67	488.98	4.20	527.61	8.00
Nd	231.61	6.12	177.51	2.58	163.86	3.81	446.48	6.23	325.74	2.56	359.50	9.11
Sm	43.73	18.51	34.92	14.34	33.76	0.75	80.68	24.74	78.10	9.04	64.94	6.66
Eu	84.20	3.25	64.42	5.20	61.78	18.43	609.38	12.06	407.66	7.20	391.19	7.33
Gd	46.81	13.14	33.69	11.02	37.29	3.87	99.06	15.36	77.31	19.46	71.77	13.22
Tb	40.67	10.02	29.55	14.50	36.09	6.41	97.02	34.44	64.84	16.34	60.59	7.27
Dy	62.93	10.69	43.16	7.18	44.50	12.83	93.64	5.49	83.35	12.40	83.35	14.20
Ho	49.80	10.25	36.16	15.06	33.35	20.71	106.54	49.71	58.44	11.20	58.98	12.53
Er	48.73	26.76	34.37	8.05	37.32	12.70	96.94	22.11	57.78	24.18	58.59	12.00
Tm	26.61	6.64	19.38	17.28	19.25	13.04	36.35	18.83	26.54	15.22	23.34	19.72
Yb	31.45	18.23	27.61	14.07	25.14	28.85	52.37	13.44	33.54	13.33	37.86	29.69
Lu	26.67	18.15	18.66	21.52	23.00	15.30	29.35	21.57	30.28	47.18	23.61	38.78

	Not diluted sample, condition 2						Not diluted sample, condition 3					
	*Samp*1		*Samp*2		*Samp*3		*Samp*1		*Samp*2		*Samp*3	
	CPS	*RSD%*	*CPS*	*RSD%*	*CPS*	*RSD%*	*CPS*	*RSD%*	*CPS*	*RSD%*	*CPS*	*RSD%*
La	2706.81	5.16	2126.44	11.39	2488.27	2.06	2605.77	2.49	1951.89	2.60	2182.97	3.88
Ce	4834.62	4.70	3737.43	1.86	4280.49	2.64	4854.76	9.57	3358.42	5.66	3948.26	4.04
Pr	693.59	3.86	485.26	7.54	610.32	4.70	665.55	2.29	479.62	9.97	566.38	8.72
Nd	515.62	2.45	361.11	6.71	420.08	0.99	440.27	4.07	333.35	8.91	392.28	9.05
Sm	90.75	13.44	71.92	18.24	83.16	13.89	86.90	7.48	70.09	2.60	79.45	0.57
Eu	488.40	13.85	388.87	7.62	403.10	3.65	480.07	5.29	359.54	5.96	368.72	3.84
Gd	107.82	5.61	81.53	10.05	88.88	12.16	102.37	8.10	87.52	0.08	84.81	6.32
Tb	85.95	5.33	65.82	13.99	75.60	18.12	74.65	4.22	66.47	5.01	66.02	7.12
Dy	111.08	13.19	84.25	16.56	83.82	14.30	105.65	6.31	84.13	11.23	86.43	3.62
Ho	87.90	14.58	61.94	13.74	71.59	16.86	82.85	6.75	65.91	4.05	69.98	9.69
Er	90.76	18.55	64.18	20.95	72.97	6.94	78.47	3.07	62.18	5.79	66.54	5.42
Tm	41.25	24.29	29.97	13.23	33.63	9.89	39.29	9.90	36.64	4.31	31.12	8.64
Yb	45.37	32.45	48.55	32.50	36.25	6.29	62.29	5.66	40.01	9.87	37.58	8.46
Lu	38.23	18.17	26.84	11.96	29.22	14.48	34.25	4.91	28.06	10.76	27.91	6.80

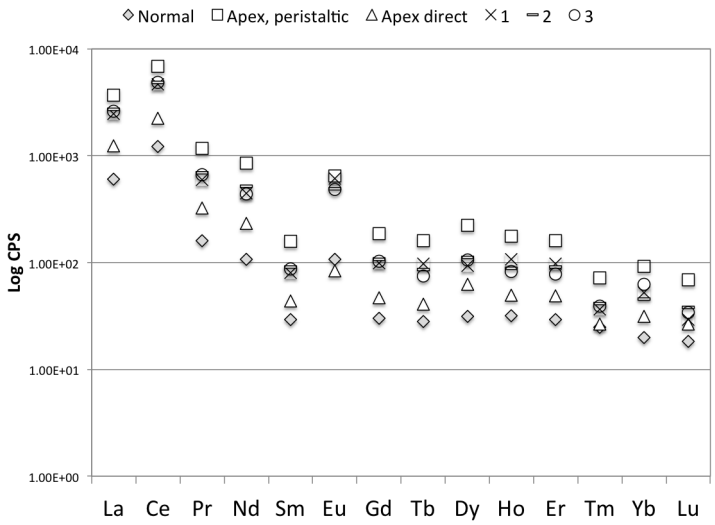


Figure 6.1: Signal comparison REEs analysis tests.

Detection limits

Detection limits were calculated for the monitored elements, according to equation 4.1 (citare LoD equazione.) and data above detection limits are signed as ¡LoD. Open vessel digestion procedure revealed the highest detection limits, especially concerning methods that used watch glasses on the tubes during heating process. Microwave digestion procedures revealed lower LOD, In table X and in table Y and Z are listed LoDs calculated for the different methods. Using UPA Acid for digestion can lower LODs up to ten times for some elements.

Recovery

Wheat flour was used as SRM. Each digestion run, both open vessel and microwave, ad at list one SRM. Table ... and table... reports the percentage recovery, calculated referring to the certificate of analysis furnished with the reference material. Procedure B revealed the better recovery compared with the other open vessel procedure. Microwave digestion test (numerare) instead had very high recovery for the most of the elements, with the exception of test (NUMERO TEST), that was performed after a double microwave cycle cleaning. This revealed a methodological error, due to the fact that was used always the same vessel for SRM digestion, leading to analytes accumulation that a single washing steps was not able to reduce. Good recovery instead was calculated in procedure ... Sodium recovery was in any case double than expected and Aluminium recovery resulted very low, around 50%.

Table 6.5: Detection limits [ng g^{-1}] Open vessel digestion methods.

Element	Mass	Acquisition mode	Procedure								
			A	*B*	*B′*	*C*	*C′*	*D*	*D′*	*E*	*F*
Li	7	*nm*	1.2	0.2	5	0.2	1.3	0.4	–	0.21	2.5
Be	9	*nm*	0.7	0.4	0.2	0.4	0.2	0.6	–	0.21	0.9
Al	27	*He*	395	743	162	144	214	172	202	347	1118
V	51	*He*	0.6	0.7	0.06	1.8	0.1	0.2	0.2	1.7	2.5
Cr	53	*He*	38	0.8	5	15	20	13	10	4	12
Mn	55	*nm*	6	5	46	4	3737	4	123	0.8	8
Mn	55	*He*	4	26	8	89	5675	0.4	146	58	8
Fe	56	*He*	1071	92	44	118	5105	49	203	441	587
Fe	57	*He*	466	107	164	158	4378	71	113	78	554
Co	59	*nm*	26	3	0.4	19	13	8	0.4	0.008	40
Co	59	*He*	19	2.3	0.04	16	16	11	1.0	1.5	35
Ni	60	*nm*	28	0.9	40	4	151	15	11	35.2	22
Ni	60	*He*	69	13	9	7	137	12	7	37.2	6
Cu	63	*nm*	15	4	30	5	619	17	21	1.9	7
Cu	63	*He*	34	4	12	14	549	14	12	9	2.2
Cu	65	*nm*	11	6	39	6	632	18	22	14	5
Cu	65	*He*	27	1.7	14	10	553	17	10	1.1	4
Zn	66	*nm*	222	10	189	26	4573	51	121	67	111
Zn	66	*He*	372	47	208	96	4054	73	79	109	74
Zn	68	*nm*	209	7	199	38	4397	55	118	72	113
Zn	68	*He*	355	18	174	128	3841	61	64	94	45
Ga	69	*nm*	0.2	2.0	0.4	2.1	8	0.8	0.3	0.7	2.2
As	75	*nm*	5	0.8	6	1.5	4	1.2	0.5	5	1.8
As	75	*He*	13	2.5	0.8	0.4	4	1.8	0.5	5	5
Se	77	*He*	155	18	40	2.7	6	16	57	13	48
Se	78	*He*	697	44	22	87	232	77	87	524	35
Se	82	*He*	164	6	31	41	26	13	80	45	109
Rb	85	*nm*	1.0	2.0	5	1.0	232	1.1	12	0.5	4
Sr	88	*nm*	14	–	4	3.6	172	–	7	1.5	3
Mo	95	*nm*	9	1.5	4	9.5	252	3	9	6	170
Ag	107	*nm*	14	3.7	2.7	3.3	20	7	1.3	4	40
Cd	111	*nm*	1.7	0.1	1.4	0.10	3	0.5	1.2	0.9	1.6
Sb	121	*nm*	0.6	0.4	0.4	0.24	0.8	1.5	0.3	4	2.6
Ba	137	*nm*	14	52.8	10	50	296	12	20	36	11
Pb	204	*nm*	9	27.5	8	50	33	3	1.0	4	19059
Tl	205	*nm*	0.4	0.2	0.2	0.28	0.1	1.0	0.0	0.3	2.3
Pb	206	*nm*	5	8.3	14	4.8	4	2.4	2.0	15	91
Pb	207	*nm*	7	9.8	32	2.60	0.5	1.4	5	42	90
Pb	208	*nm*	4	7.8	32	4.3	0.7	1.2	2.0	41	89
Bi	209	*nm*	0.8	0.2	0.9	0.18	0.2	1.0	3	1.1	2.1

Table 6.6: Procedurals blanks averages [$\mathrm{ng\,g^{-1}}$] Open vessel digestion methods.

Element	Mass	Acquisition mode	Procedure								
			A	B	B'	C	C'	D	D'	E	F
Li	7	nm	497.8 ± 0.4	495.8 ± 0.1	496 ± 2	492.45 ± 0.07	492.0 ± 0.4	492.2 ± 0.1	–	492.05 ± 0.07	495.8 ± 0.8
Be	9	nm	381.3 ± 0.2	380.8 ± 0.1	380.9 ± 0.1	380.6 ± 0.1	380.5 ± 0.1	380.8 ± 0.2	–	380.75 ± 0.07	381.2 ± 0.3
Al	27	He	647 ± 132	736 ± 248	966 ± 54	905 ± 48	1139 ± 71	607 ± 57	1126.5 ± 67	682 ± 116	2804 ± 373
V	51	He	3.3 ± 0.2	3.1 ± 0.2	3.59 ± 0.02	3.6 ± 0.6	3.2 ± 0.0	4.0 ± 0.1	3.059 ± 0.1	3.4 ± 0.6	7.0 ± 0.8
Cr	53	He	839 ± 13	843.2 ± 0.3	834 ± 2	848 ± 5	838 ± 7	827 ± 4	836.5 ± 3	838 ± 1	874 ± 4
Mn	55	nm	528 ± 2	537 ± 2	575 ± 15	539 ± 1	1471 ± 1246	525 ± 1	574.95 ± 41	539.2 ± 0.3	539 ± 3
Mn	55	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	
Fe	56	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	695 ± 196
Fe	57	He	524 ± 155	254 ± 36	202 ± 55	208 ± 53	1451 ± 1459	43 ± 24	304 ± 38	196 ± 26	875 ± 185
Co	59	nm	11 ± 9	10 ± 1	5.5 ± 0.1	10 ± 6	8 ± 4	7 ± 3	4.7655 ± 0.1	4.963 ± 0.003	14 ± 13
Co	59	He	8 ± 6	8.3 ± 0.8	3.63 ± 0.01	8 ± 5	7 ± 5	7 ± 4	3.2375 ± 0.3	3.4 ± 0.5	11 ± 12
Ni	60	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Ni	60	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Cu	63	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Cu	63	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Cu	65	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Cu	65	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Zn	66	nm	132 ± 74	$< LoD$	73 ± 63	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Zn	66	He	133 ± 124	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Zn	68	nm	142 ± 70	$< LoD$	82 ± 66	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Zn	68	He	116 ± 118	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Ga	69	nm	4.9 ± 0.1	4.2 ± 0.7	4.8 ± 0.1	4.8 ± 0.7	7 ± 3	4.3 ± 0.3	4.0285 ± 0.1	4.3 ± 0.2	5.8 ± 0.7
As	75	nm	24 ± 2	17.5 ± 0.3	19 ± 2	13.1 ± 0.5	11 ± 1	9.5 ± 0.4	9.6755 ± 0.2	11 ± 2	15.3 ± 0.6
As	75	He	25 ± 4	16.3 ± 0.8	16.2 ± 0.3	12.8 ± 0.1	11 ± 1	11.7 ± 0.6	9.6655 ± 0.2	11 ± 2	14 ± 2
Se	77	He	42 ± 52	9 ± 6	14 ± 13	22.3 ± 0.9	$< LoD$	$< LoD$	–	$< LoD$	$< LoD \pm 16$
Se	78	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	1 ± 12
Se	82	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Rb	85	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Sr	88	nm	$< LoD$	–	$< LoD$	$< LoD$	$< LoD$	–	$< LoD$	$< LoD$	$< LoD$
Mo	95	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Ag	107	nm	59 ± 5	10.3 ± 1.2	12.1 ± 0.9	5.4 ± 1.1	8 ± 7	4 ± 2	3.25 ± 0.4	4 ± 1	114 ± 13
Cd	111	nm	6.9 ± 0.6	6.5 ± 0.0	6.8 ± 0.5	6.08 ± 0.03	7 ± 1	6.5 ± 0.2	6.153 ± 0.4	6.3 ± 0.3	7.6 ± 0.5
Sb	121	nm	9.7 ± 0.2	8.0 ± 0.1	8.2 ± 0.1	7.42 ± 0.08	7.0 ± 0.3	8.0 ± 0.5	7.3365 ± 0.1	8 ± 1	9.9 ± 0.9
Ba	137	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	–
Pb	204	nm	37 ± 3	44.1 ± 9.2	47 ± 3	89 ± 17	16 ± 11	1 ± 1	10.223 ± 0.3	14 ± 1	5552 ± 6353
Tl	205	nm	4.0 ± 0.1	3.7 ± 0.1	3.7 ± 0.1	3.61 ± 0.09	3.6 ± 0.0	4.3 ± 0.3	3.564 ± 0.0	3.9 ± 0.1	4.8 ± 0.8
Pb	206	nm	32 ± 2	35.5 ± 2.8	34 ± 5	9.1 ± 1.6	7 ± 1	5.6 ± 0.8	13.9 ± 0.7	16 ± 5	41 ± 30
Pb	207	nm	30 ± 2	34.1 ± 3.3	31 ± 11	7.74 ± 0.87	12.0 ± 0.2	11.7 ± 0.5	22.825 ± 2	23 ± 14	40 ± 30
Pb	208	nm	30 ± 1	34.4 ± 2.6	32 ± 11	9.2 ± 1.4	13.4 ± 0.2	11.6 ± 0.4	24.125 ± 0.7	23 ± 14	40 ± 30
Bi	209	nm	5.3 ± 0.3	4.7 ± 0.1	5.9 ± 0.3	4.60 ± 0.06	4.8 ± 0.1	6.0 ± 0.3	5.609 ± 1	5.2 ± 0.4	5.8 ± 0.7

Table 6.7: Recovery [%] Open vessel digestion methods.

Element	Mass	Acquisition mode	Procedure								
			A	B	B'	C	C'	D	D'	E	F
Al	27	He	12	17	22	69	37	40	37	46	9
V	51	He	21	34	41	49	69	70	96	77	19
Mn	55	nm	41	97	99	135	102	114	128	106	62
Mn	55	He	53	96	92	233	139	133	151	119	64
Fe	56	He	50	90	86	210	118	115	218	107	61
Fe	57	He	45	81	77	129	110	107	129	96	55
Co	59	nm	$< LoD$	35	23	190	110	109	667	$< LoD$	$< LoD$
Co	59	He	$< LoD$	15	9	168	$< LoD$	$< LoD$	648	$< LoD$	$< LoD$
Cu	63	nm	55	97	101	136	94	104	122	105	61
Cu	63	He	48	85	82	138	117	110	125	103	56
Cu	65	nm	55	98	102	137	94	104	122	105	61
Cu	65	He	48	85	80	137	116	110	126	102	56
Zn	66	nm	69	110	118	148	105	119	137	122	74
Zn	66	He	61	96	95	149	129	123	139	118	69
Zn	68	nm	66	107	114	144	102	116	133	118	72
Zn	68	He	57	88	88	138	119	115	130	110	64
As	75	nm	90	100	79	84	182	169	51	98	73
As	75	He	70	100	58	74	196	120	58	119	47
Se	77	He	72	94	101	148	120	105	125	117	75
Se	78	He	75	116	120	159	106	92	107	135	82
Se	82	He	80	127	120	170	129	120	136	136	86
Rb	85	nm	52	96	98	121	79	89	103	106	59
Mo	95	nm	55	95	99	125	78	88	112	106	71
Pb	204	nm	42	70	422	$< LoD$	74	86	29	$< LoD$	$< LoD$
Pb	206	nm	35	66	386	95	71	92	30	$< LoD$	28
Pb	207	nm	37	61	403	173	111	147	279	$< LoD$	37
Pb	208	nm	38	66	395	175	123	150	276	$< LoD$	34

Table 6.8: Detection limits [ng g^{-1}] Microwave digestion methods.

El.	Mass	Acq. mode	Procedure						
			$MW-1$	$MW-2$	$MW-3$	$MW-4$	$MW-5$	$MW-6$	$MW-7$
Li	7	nm	0.016	0.011	0.0017	0.0012	0.0016	0.0025	0.0018
Be	9	nm	0.014	0.006	0.0010	0.0007	0.0022	0.0008	0.0004
Na	23	nm	2.3	5	3	1.0	1.1	1.7	2.1
Mg	24	nm	2.7	4	2.8	7	3	6	1
Al	27	He	0.5	1.6	0.28	0.5	0.5	0.5	0.3
K	39	nm	1.2	19	11	12	9	19	11
Ca	43	nm	5	18	2.7	6	1.9	4.4	0.6
Ca	43	He	2	3	1.1	1.3	0.7	1.6	0.3
Ca	43	H_2	4	11	1.3	8	3	4	0.008
Ca	44	nm	7	21	4	8	0.3	5.8	3.1
Ca	44	He	3	7	0.4	2.5	1.7	1.9	0.5
Ca	44	H_2	7	16	1.3	13	5	5	1.5
V	51	He	0.006	0.009	0.002	0.0005	0.0009	0.0015	0.003
Cr	52	He	0.21	0.03	0.05	0.016	0.003	0.007	0.010
Cr	53	He	0.22	0.06	0.06	0.013	0.014	0.016	0.03
Mn	55	nm	0.03	0.26	0.07	0.14	0.07	0.11	0.09
Mn	55	He	0.012	0.20	0.16	0.15	0.14	0.20	0.03
Fe	56	He	1.1	0.6	0.7	0.5	0.3	0.5	0.25
Fe	56	H_2	1.2	1.1	0.4	2.7	0.8	0.8	0.13
Fe	57	He	1.1	0.5	0.9	0.4	0.4	0.4	0.4
Co	59	nm	0.07	0.02	0.018	0.010	0.01	0.0022	0.0012
Co	59	He	0.09	0.012	0.016	0.010	0.01	0.005	0.0010
Ni	60	nm	0.09	0.17	0.27	0.026	0.06	0.003	0.003
Ni	60	He	0.11	0.19	0.21	0.03	0.06	0.005	0.009
Cu	63	nm	0.05	0.08	0.13	0.035	0.00	0.03	0.04
Cu	63	He	0.03	0.08	0.09	0.023	0.04	0.03	0.007
Cu	65	nm	0.06	0.08	0.13	0.022	0.016	0.021	0.017
Cu	65	He	0.04	0.08	0.08	0.026	0.04	0.04	0.008
Zn	66	nm	0.28	0.16	0.26	0.14	0.18	0.13	0.08
Zn	66	He	0.4	0.10	0.09	0.14	0.3	0.21	0.028
Zn	68	nm	0.3	0.16	0.28	0.14	0.21	0.11	0.04
Zn	68	He	0.3	0.10	0.10	0.13	0.4	0.16	0.008
Ga	69	nm	0.02	0.009	0.0014	0.003	0.0004	0.0009	0.0016
As	75	nm	0.02	0.005	0.0024	0.0009	0.000013	0.0015	0.0014
As	75	He	0.003	0.008	0.0021	0.004	0.002	0.003	0.0006
Se	77	He	0.07	0.05	0.027	0.04	0.09	0.012	0.03
Se	77	H_2	0.03	0.04	0.04	0.14	0.03	0.04	0.004
Se	78	He	0.19	0.3	0.15	0.15	0.20	0.25	0.05
Se	78	H_2	0.03	0.03	0.020	0.022	0.023	0.019	0.03
Se	82	He	0.12	0.11	0.11	0.18	0.12	0.05	0.08
Se	82	H_2	0.14	0.04	0.03	0.6	0.030	0.4	0.3
Rb	85	nm	0.02	0.02	0.005	0.007	0.002	0.12	0.010
Sr	88	nm	0.04	0.07	0.0005	0.04	0.015	0.04	0.005
Mo	95	nm	0.02	0.02	0.06	0.12	0.005	0.008	0.03
Ag	107	nm	0.02	0.008	0.0009	0.0006	0.0009	0.0006	0.0003
Cd	111	nm	0.015	0.008	0.0012	0.0007	0.0004	0.0012	0.004
Sb	121	nm	0.014	0.007	0.0006	0.00011	0.0002	0.00006	0.0004
Ba	137	nm	0.04	0.06	0.04	0.09	0.012	0.018	0.05
Pb	204	nm	0.021	0.011	0.013	0.07	0.013	0.03	0.03
Tl	205	nm	0.016	0.007	0.0006	0.00014	0.0003	0.0009	0.00008
Pb	206	nm	0.016	0.006	0.006	0.0022	0.013	0.007	0.003
Pb	207	nm	0.016	0.009	0.007	0.0019	0.015	0.008	0.005
Pb	208	nm	0.018	0.008	0.006	0.0013	0.014	0.008	0.006
Bi	209	nm	0.015	0.007	0.0012	0.0004	0.0008	0.0004	0.00013

Table 6.9: Procedurals blanks averages [ng g^{-1}] Microwave digestion methods.

El.	Mass	Acq. mode	Procedure						
			$MW-1$	$MW-2$	$MW-3$	$MW-4$	$MW-5$	$MW-6$	$MW-7$
Li	7	nm	0.013 ± 0.005	0.012 ± 0.004	0.0034 ± 0.0006	0.0022 ± 0.0004	0.0036 ± 0.0005	0.0031 ± 0.0008	0.0031 ± 0.0006
Be	9	nm	0.007 ± 0.005	0.004 ± 0.002	$< LoD$	0.0003 ± 0.0002	$< LoD$	0.0006 ± 0.0003	0.0004 ± 0.0001
Na	23	nm	1.5 ± 0.8	5 ± 1.7	3 ± 1	2.9 ± 0.3	7.5 ± 0.4	7.1 ± 0.6	8.3 ± 0.7
Mg	24	nm	1.8 ± 0.9	11 ± 1.4	1.6 ± 0.9	6 ± 2	4 ± 1	5 ± 2	8 ± 0
Al	27	He	1.4 ± 0.2	1.8 ± 0.5	1.30 ± 0.09	1.0 ± 0.2	1.2 ± 0.2	0.7 ± 0.2	0.7 ± 0.1
K	39	nm	$< LoD$	15 ± 6.4	6 ± 4	13 ± 4	11 ± 3	17 ± 6	26 ± 4
Ca	43	nm	7 ± 2	16 ± 6	3.6 ± 0.9	5 ± 2	4.1 ± 0.6	4.6 ± 1.5	4.5 ± 0.2
Ca	43	He	2 ± 1	4 ± 1	1.1 ± 0.4	1.5 ± 0.4	1.2 ± 0.2	1.2 ± 0.5	1.4 ± 0.1
Ca	43	H_2	4 ± 1	10 ± 4	2.5 ± 0.4	5 ± 3	3 ± 1	3 ± 1	3.278 ± 0.003
Ca	44	nm	5 ± 2	20 ± 7	3 ± 1	5 ± 3	4.8 ± 0.1	5.6 ± 1.9	5.6 ± 1.0
Ca	44	He	2 ± 1	6 ± 2	1.3 ± 0.1	2.2 ± 0.8	1.9 ± 0.6	1.9 ± 0.6	1.8 ± 0.2
Ca	44	H_2	$< LoD$	10 ± 5	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$
V	51	He	0.005 ± 0.002	0.005 ± 0.003	$< LoD$	$< LoD$	0.0009 ± 0.0003	$< LoD$	0.006 ± 0.001
Cr	52	He	0.11 ± 0.07	0.07 ± 0.01	0.07 ± 0.02	0.015 ± 0.005	0.012 ± 0.001	0.012 ± 0.002	0.022 ± 0.003
Cr	53	He	0.12 ± 0.07	0.08 ± 0.02	0.08 ± 0.02	0.008 ± 0.004	0.011 ± 0.005	0.008 ± 0.005	0.02 ± 0.01
Mn	55	nm	0.03 ± 0.01	0.18 ± 0.09	0.06 ± 0.02	0.14 ± 0.05	0.11 ± 0.02	0.12 ± 0.04	0.24 ± 0.03
Mn	55	He	0.015 ± 0.004	0.14 ± 0.066	$< LoD$	0.13 ± 0.05	0.16 ± 0.05	0.11 ± 0.07	0.24 ± 0.01
Fe	56	He	1.4 ± 0.4	1.9 ± 0.2	1.1 ± 0.2	0.5 ± 0.2	0.6 ± 0.1	0.5 ± 0.2	0.51 ± 0.08
Fe	56	H_2	1.6 ± 0.4	2.2 ± 0.4	1.4 ± 0.1	$< LoD$	0.7 ± 0.3	0.4 ± 0.3	0.50 ± 0.04
Fe	57	He	1.2 ± 0.4	1.9 ± 0.2	0.9 ± 0.3	0.3 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Co	59	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Co	59	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Ni	60	nm	0.24 ± 0.03	0.25 ± 0.06	0.36 ± 0.09	0.046 ± 0.009	0.05 ± 0.02	0.042 ± 0.001	0.048 ± 0.001
Ni	60	He	0.23 ± 0.04	0.23 ± 0.06	0.32 ± 0.07	0.02 ± 0.01	0.02 ± 0.02	0.013 ± 0.002	0.023 ± 0.003
Cu	63	nm	0.14 ± 0.02	0.17 ± 0.03	0.20 ± 0.04	0.082 ± 0.012	0.07 ± 0.00	0.08 ± 0.01	0.13 ± 0.01
Cu	63	He	0.09 ± 0.01	0.12 ± 0.03	0.14 ± 0.03	0.024 ± 0.008	0.03 ± 0.01	0.02 ± 0.01	0.060 ± 0.002
Cu	65	nm	0.13 ± 0.02	0.15 ± 0.03	0.18 ± 0.04	0.058 ± 0.007	0.051 ± 0.005	0.054 ± 0.007	0.093 ± 0.006
Cu	65	He	0.09 ± 0.01	0.11 ± 0.03	0.14 ± 0.03	0.019 ± 0.009	0.02 ± 0.01	0.02 ± 0.01	0.054 ± 0.003
Zn	66	nm	0.31 ± 0.09	0.39 ± 0.05	0.38 ± 0.09	0.11 ± 0.05	0.13 ± 0.06	0.14 ± 0.04	0.22 ± 0.03
Zn	66	He	0.3 ± 0.1	0.38 ± 0.0	0.37 ± 0.03	0.15 ± 0.05	0.2 ± 0.1	0.18 ± 0.07	0.257 ± 0.009
Zn	68	nm	0.3 ± 0.1	0.38 ± 0.1	0.39 ± 0.09	0.12 ± 0.05	0.14 ± 0.07	0.14 ± 0.04	0.23 ± 0.01
Zn	68	He	0.3 ± 0.1	0.36 ± 0.0	0.35 ± 0.03	0.13 ± 0.04	0.2 ± 0.1	0.15 ± 0.05	0.229 ± 0.003
Ga	69	nm	0.04 ± 0.01	0.041 ± 0.00	0.0353 ± 0.0005	0.036 ± 0.001	0.0358 ± 0.0001	0.0340 ± 0.0003	0.0348 ± 0.0005
As	75	nm	0.02 ± 0.01	0.015 ± 0.00	0.0106 ± 0.0008	0.0103 ± 0.0003	0.009658 ± 0.000004	0.0096 ± 0.0005	0.0085 ± 0.0005
As	75	He	0.008 ± 0.001	0.007 ± 0.003	0.0017 ± 0.0007	0.002 ± 0.001	0.001 ± 0.001	0.002 ± 0.001	0.0009 ± 0.0002
Se	77	He	0.07 ± 0.02	0.06 ± 0.02	0.038 ± 0.009	0.04 ± 0.01	0.06 ± 0.03	0.051 ± 0.004	0.05 ± 0.01
Se	77	H_2	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.07 ± 0.05	0.06 ± 0.01	0.04 ± 0.01	0.056 ± 0.001
Se	78	He	0.15 ± 0.06	0.2 ± 0.10	0.14 ± 0.05	0.16 ± 0.05	0.16 ± 0.07	0.13 ± 0.08	0.07 ± 0.02
Se	78	H_2	0.03 ± 0.01	0.02 ± 0.01	0.027 ± 0.007	0.032 ± 0.007	0.026 ± 0.008	0.025 ± 0.006	0.03 ± 0.01
Se	82	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Se	82	H_2	0.10 ± 0.05	0.21 ± 0.01	0.20 ± 0.01	0.3 ± 0.2	0.274 ± 0.010	0.7 ± 0.1	0.5 ± 0.1
Rb	85	nm	0.05 ± 0.01	0.05 ± 0.01	0.044 ± 0.002	0.049 ± 0.002	0.049 ± 0.001	0.15 ± 0.04	0.095 ± 0.003
Sr	88	nm	0.06 ± 0.01	0.13 ± 0.02	0.0337 ± 0.0002	0.05 ± 0.01	0.053 ± 0.005	0.06 ± 0.01	0.054 ± 0.002
Mo	95	nm	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	$< LoD$	0.011 ± 0.002	0.011 ± 0.003	0.03 ± 0.01
Ag	107	nm	0.01 ± 0.01	0.005 ± 0.003	0.0007 ± 0.0003	$< LoD$	$< LoD$	0.0004 ± 0.0002	0.0003 ± 0.0001
Cd	111	nm	0.007 ± 0.005	0.005 ± 0.003	0.0010 ± 0.0004	0.0006 ± 0.0002	0.0016 ± 0.0001	0.0014 ± 0.0004	0.009 ± 0.001
Sb	121	nm	0.008 ± 0.005	0.005 ± 0.002	0.0012 ± 0.0002	0.00087 ± 0.00004	0.0007 ± 0.0001	0.00083 ± 0.00002	0.0012 ± 0.0001
Ba	137	nm	0.11 ± 0.01	0.13 ± 0.02	0.10 ± 0.01	0.12 ± 0.03	0.103 ± 0.004	0.069 ± 0.006	0.09 ± 0.02
Pb	204	nm	0.010 ± 0.007	0.006 ± 0.004	$< LoD$	$< LoD$	0.011 ± 0.004	$< LoD$	0.04 ± 0.01
Tl	205	nm	0.005 ± 0.005	0.002 ± 0.002	$< LoD$	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Pb	206	nm	0.011 ± 0.005	0.007 ± 0.002	0.003 ± 0.002	0.0015 ± 0.0007	0.005 ± 0.004	0.003 ± 0.002	0.017 ± 0.001
Pb	207	nm	0.011 ± 0.005	0.007 ± 0.003	0.003 ± 0.002	0.0014 ± 0.0006	$< LoD$	0.004 ± 0.003	0.016 ± 0.002
Pb	208	nm	0.011 ± 0.006	0.007 ± 0.003	0.003 ± 0.002	0.0010 ± 0.0004	0.0050.005	0.004 ± 0.003	0.016 ± 0.002
Bi	209	nm	0.007 ± 0.005	0.004 ± 0.002	0.0007 ± 0.0004	0.0003 ± 0.0001	0.0009 ± 0.0003	0.0005 ± 0.0001	0.00050 ± 0.00004

Table 6.10: Recovery [%] Microwave digestion methods.

El.	Mass	Acq. mode	Procedure						
			$MW-1$	$MW-2$	$MW-3$	$MW-4$	$MW-5$	$MW-6$	$MW-7$
Na	23	nm	448	376	349	430	419	331	300
Mg	24	nm	287	276	236	268	262	209	179
Al	27	He	175	149	124	192	146	116	121
K	39	nm	273	253	221	258	250	195	179
Ca	43	nm	178	196	143	168	165	131	113
Ca	43	He	41	45	34	40	38	30	27
Ca	43	H_2	116	130	96	119	113	86	99
Ca	44	nm	206	225	168	194	190	153	131
Ca	44	He	66	74	56	65	63	49	45
Ca	44	H_2	157	169	130	156	150	114	131
Mn	55	nm	319	301	261	306	291	235	204
Mn	55	He	253	243	215	283	266	199	171
Fe	56	He	240	249	212	250	231	182	429
Fe	56	H_2	309	320	262	327	298	234	579
Fe	57	He	272	283	232	282	259	207	485
Co	59	nm	–	–	–	–	–	–	247
Co	59	He	–	–	–	–	–	–	285
Cu	63	nm	261	245	209	239	235	188	162
Cu	63	He	240	233	204	236	231	178	167
Cu	65	nm	267	252	213	245	240	192	166
Cu	65	He	244	235	207	239	234	181	169
Zn	66	nm	257	253	209	240	237	195	188
Zn	66	He	223	226	193	222	219	173	174
Zn	68	nm	255	250	207	238	236	193	186
Zn	68	He	215	217	184	212	211	165	167
As	75	nm	–	137	121	175	155	115	123
As	75	He	119	115	129	174	167	105	135
Se	77	He	232	240	197	242	244	184	177
Se	77	H_2	205	214	174	220	217	175	205
Se	78	He	227	229	195	234	234	187	179
Se	78	H_2	211	215	171	222	219	169	198
Se	82	He	252	239	204	249	248	184	181
Se	82	H_2	562	590	483	601	550	419	445
Rb	85	nm	237	214	189	220	213	166	151
Mo	95	nm	291	269	238	269	265	211	180
Cd	111	nm	268	259	222	245	243	203	186

Table 6.11: Detection limits [$\mathrm{ng\,g^{-1}}$] Microwave digestion methods.

El.	Mass	Acq. mode	Procedure			
			$MW-8$	$MW-9$	$MW-10$	$MW-11$
Na	23	nm	0.3	0.8	45.5	6.9
Mg	24	nm	7	2	24	12
Al	27	He	0.5	0.3	0.7	0.5
K	39	nm	7	1.0	7	6
Ca	43	nm	18	4	43	69
Ca	43	He	2.3	1.6	8	18
Ca	43	H_2	39	12	68	166
Ca	44	nm	29	7	57	85
Ca	44	He	9	3	14	28
Ca	44	H_2	90	19	98	248
V	51	He	–	0.005	0.002	0.003
Cr	52	He	0.12	0.04	0.11	0.09
Cr	53	He	0.13	0.04	0.08	0.05
Mn	55	nm	0.012	0.011	0.018	0.020
Mn	55	He	0.012	0.008	0.003	0.022
Fe	56	He	0.9	0.4	0.4	0.8
Fe	56	H_2	2.1	0.8	1.3	2.0
Fe	57	He	0.8	0.4	0.7	1.1
Ni	60	nm	0.003	0.002	0.012	0.02
Ni	60	He	0.006	0.002	0.015	0.03
Cu	63	nm	0.25	0.04	0.16	0.20
Cu	63	He	0.19	0.04	0.10	0.24
Cu	65	nm	0.25	0.023	0.17	0.19
Cu	65	He	0.18	0.05	0.09	0.24
Zn	66	nm	0.07	0.03	0.19	0.15
Zn	66	He	0.04	0.04	0.05	0.15
Zn	68	nm	0.07	0.01	0.20	0.14
Zn	68	He	0.07	0.03	0.05	0.16
As	75	nm	0.01	0.014	0.02	0.01
As	75	He	0.02	0.003	0.03	0.01
Se	77	He	0.09	0.29	0.12	0.13
Se	77	H_2	0.16	0.10	0.25	0.15
Se	78	He	0.18	0.22	0.25	0.09
Se	78	H_2	0.05	0.17	0.09	0.06
Se	82	He	0.08	0.19	0.14	0.07
Se	82	H_2	0.17	0.36	0.20	0.45
Rb	85	nm	0.002	0.005	0.003	0.003
Sr	88	nm	0.02	0.05	0.22	0.04
Mo	95	nm	0.008	0.01	0.015	0.007
Ag	107	nm	0.003	0.00	0.003	0.005
Ba	137	nm	0.009	0.02	0.04	0.02

Table 6.12: Recovery [%] Microwave digestion methods.

El.	Mass	Acq. mode	Procedure			
			$MW-8$	$MW-9$	$MW-10$	$MW-11$
Na	23	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Mg	24	nm	9 ± 2	6.8 ± 0.8	$< LoD$	$< LoD$
Al	27	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$
K	39	nm	$< LoD$	11.1 ± 0.3	14 ± 2	12 ± 2
Ca	43	nm	38 ± 6	29 ± 1	48 ± 14	$< LoD$
Ca	43	He	7.3 ± 0.8	5.54 ± 0.5	8 ± 3	$< LoD$
Ca	43	H_2	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Ca	44	nm	49.33 ± 9.61	38 ± 2	63 ± 19	$< LoD$
Ca	44	He	14 ± 3	10.78 ± 1	15 ± 5	$< LoD$
Ca	44	H_2	$< LoD$	$< LoD$	$< LoD$	$< LoD$
V	51	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Cr	52	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Cr	53	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Mn	55	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Mn	55	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Fe	56	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Fe	56	H_2	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Fe	57	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Ni	60	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Ni	60	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Cu	63	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Cu	63	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Cu	65	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Cu	65	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Zn	66	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Zn	66	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Zn	68	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Zn	68	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$
As	75	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$
As	75	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Se	77	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Se	77	H_2	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Se	78	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Se	78	H_2	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Se	82	He	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Se	82	H_2	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Rb	85	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Sr	88	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Mo	95	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Ag	107	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$
Ba	137	nm	$< LoD$	$< LoD$	$< LoD$	$< LoD$

Table 6.13: Detection limits [ng g^{-1}] Microwave digestion methods.

El.	Mass	Acq. mode	Procedure			
			$MW-8$	$MW-9$	$MW-10$	$MW-11$
Na	23	nm	176	214	297	256
Mg	24	nm	107	119	140	127
Al	27	He	50	53	55	55
K	39	nm	111	120	137	126
Ca	43	nm	58	64	70	72
Ca	43	He	16	16	16	17
Ca	43	H_2	95	120	137	117
Ca	44	nm	72	83	87	90
Ca	44	He	26	27	27	29
Ca	44	H_2	163	189	218	189
Mn	55	nm	94	106	128	108
Mn	55	He	95	93	103	96
Fe	56	He	93	102	110	111
Fe	56	H_2	150	147	167	161
Fe	57	He	93	97	103	106
Cu	63	nm	37	44	52	42
Cu	63	He	62	66	79	76
Cu	65	nm	63	68	78	66
Cu	65	He	74	76	88	86
Zn	66	nm	93	96	110	99
Zn	66	He	91	89	98	96
Zn	68	nm	89	91	105	95
Zn	68	He	83	81	90	89
Se	77	He	55	61	50	47
Se	77	H_2	–	–	–	–
Se	78	He	64	62	65	56
Se	78	H_2	–	–	–	–
Se	82	He	65	65	51	55
Se	82	H_2	59	115	263	69
Rb	85	nm	93	103	120	111

6.4 Method application for a traceability study

6.4.1 Samples description

Samples used for traceability study As mentioned above, samples analysed in this study were furnished by CRA. They provided us kernels of 4 barley genotypes samples (Aldebaran, Braemar, Kangoo and Concerto) grown up in replicate field trials. (Fig. 6.2). Those samples are the result of national tests on malt quality barley in 2009-2010 [[**INFOAgrario**]]. Aldebaran a six rowed, zoothecnic barley type, having a high protein content. Braemar, concerto Kangoo instead, have two-rowed ears and are specifics for malt production, having low protein levels. In particular Concerto highly suitable for both brewing and distilling, and his malt is appreciated in Italian microbrewery. On a large set 13 cultivar, Aldebaran was chosen as reference zootechnic type barley and the others were randomly chosen between malt type samples for method application [**CRA2004**] [**INFOAgrario**].

Barley cultivated in one of the fiel trial (Tolentino) was malted in CRA laboratory, with an automatic Micromalting System (Phoenix Biosistem, South Australia) as described in Gianinetti et al (2005). With malt, there were also trashes, derived from malt production, due to rootlets, hulls and sprouts removal after kilning step.

Sites description The cultivation sites were distributed longitudinally along the Italian peninsula, and the main islands.

- Fiorenzuola d'arda (PC): Town in the Northern Italy placed in the Po valley at 82MASL. Barley here was grown up in spring, when the average rainfall is 226 mm and the temperature vary between 6 °Cand 17 °C.
- Tolentino (MC): Located at 230 m above sea level, in centre-east Italy, in Marche region. Barley was grown up both in spring and fall. In spring rainfall average is around 210.9 mm and the temperature range between 9°C and 16°C. In fall the temperature are a little bit higher, varying between 18.1°C and 12.2°C, ant the rainfall average is 220.9 mm.
- Larino (CB): Located in Molise region, at 400 MASL. Barleys were seeded in fall, when the average temperature ranged between 9.7°C and 16.6°C and the average seasonal rainfall is around 161mm.
- Foggia: Southern Italy site in Puglia region, placed at 76 MASL. Barley here was seeded in fall, when the rainfall is around 152mm and the temperature vary between 11°C and 22.3°C.
- Libertinia(CT): Village at 267 m above sea level, placed in middle of Sicily island. The cultivar was seeded in fall, when the average temperature rages between 13 and 22°C ant the rainfall reaches averages values of 188mm.

- Ussana (CA): Placed in the south of Sardinia, at 97MASL. Barley was seeded in fall, when the rainfall average rangse around 173mm and the temperature vary between 8.9°C 18.6°C.

Figure 6.2: Barley production sites.

Chapter 7

Experimental

7.1 Barley malt and trashes multielemental analysis

7.1.1 Sample preparation

Cereals samples were microwave digested but first they were milled and homogenised with a mechanical ball mill (Retsch), equipped with Teflon vessels.

The samples were weighted into pre-cleaned Tefon digestion vessels (0.5 g barley-malt, 0.25 g trashes) and6 mL of HNO_3 doubly distilled and 4 mL of H_2O_2 UpA were added. Each sample was digested in duplicate, and 0.5 g of SRM-Wheat Flour was digested too. Apart from samples and SRM, for each digestion run three blanks were prepared. The samples were microwaved digested using digestion program number 3. With a power of 15000W the temperature was enhanced to 100°C in 10 minutes, and was kept for other 10 minutes. The temperature was then carried to 200° in the next 10 minutes, and was kept there 15 minutes, before start ventilation. Once cooled, the solutions were transferred to PP vessels and diluted with ultrapure water up to 50 mL. Samples were stored at -20°C until analysis. Before ICP-MS analysis, the digested samples were opportunely diluted, while rare earth elements analysis was performed on samples as it is, according to the procedure previously tested.

7.1.2 ICP-QMS analysis

Multielemental measurements were carried out with an inductively coupled plasma quadrupole mass spectrometer (ICP-MS) (Agilent 7500 ORS – Agilent Technologies Inc. Santa Clara, California), equipped with an octapole reaction system (ORS). The ion source was composed of a Peltier-cooled quartz spray chamber, and a quartz torch with a quartz injector tube. The instrumental performance was monitored before starting every analysis session, acquiring a multi element tuning solution, containing about 1 ng g^{-1} of 7Li, 89Y , 205Tl and 140Ce. The instrumental performance optimization was carried out adjusting torch alignment, nebulizer gas flow

rate, RF power and lens voltages. An AUTOSAMPLER was used for solutions acquisition. In order to correct the temporal variations in signal intensity, a platinum and rhodium $10\,\mathrm{ng\,g^{-1}}$ standard solution was added on-line. To lower as much as possible isobaric interferences the reaction cell was used , with He and H_2 as reaction gas.

7.2 Data evaluation

Analytical data were exported as quantitative values, result of the average of 3 acquisitions. Elements concentrations were calculated by instrument software on the basis of calibration curves, after signal correction by internal standard intensity. Concentration data were manually blank corrected and the samples composition were calculated taking into account dilution steps and sample weight. Data under detection limits In data evaluation was included only the isotope that gives better result in term of repeatability and recovery. Aluminium and Sodium were excluded from data handling because of their bad recovery. For Aluminium the recovery calculated did not exceeded 60% while, for sodium, recovery was almost double compared to the certificate values. (rif tabella). Calcium had a bad recovery, and the acquisition with the reaction cell did not improve the result. Even if the calculated recovery for ^{44}Ca acquired with H_2 was good, it could not be considered because of his high standard deviation. The bad reproducibility can be clearly seen in term or repetability of the measurement. Also Chromo and Silver repeatability values were very low, so they were rejected. (rif tabella)..

Elemental composition showed a very high variability in term of concentration. Magnesium and potassium are highly concentrated in barley, malt and trashes samples, and their amount goes from 0.1 to 0.5 % w/w. Manganese, Iron, Copper, Zinc Rubidium, Strontium and Barium were found to be at trace level. Lithium, Vanadium, Cobalt, Nickel, Gallium, Arsenic, and REEs had concentration varying from about $900\,\mathrm{ng\,g^{-1}}$ to a few $\mathrm{ng\,g^{-1}}$.

7.3 Barley malt and trashes Sr isotope ratio analy

7.3.1 Strontium/matrix separation

Interfering species due to the sample matrix disturbs isotopic ratio 87Sr/86Sr measurement. Strontium isotopic analysis is commonly preceded by a matrix separation preanalytical step, to lower as much as possible isobaric interference on mass 87 due to Rb. Moreover, sample treatment, can reduce interferences due to other elements (as Ca, or P), reducing matrix influence on Sr detection. According to Swoboda et al. [2008] a separation procedure was optimized in order to divide Sr from sample matrix before isotopic analysis. Sr/matrix separation consists in an extraction chromatography, performed with a Sr-Spec resin. The resin, a crown-ether (bis-t-butyl-cis- dicyclohexano-18-crown-6) absorbed on inert substrate, has the capability to hold and release Sr depending on pH [TRISKEM:Eichrom, 2007], washing away Rb (and other matrix

Table 7.1: Detection limits[ng g^{-1}], quantification limits[ng g^{-1}] and repeatability of method.

Element	Mass	Acq. mode	LoD	LoQ	Rep %
Li	7	nm	0.013	0.042	90
Be	9	nm	0.03	0.09	–
Na	23	nm	3.0	9.9	92
Mg	24	nm	50	166	94
Al	27	He	0.9	3.0	88
K	39	nm	16	53	97
Ca	43	nm	43	145	51
Ca	43	He	43	144	55
Ca	43	H_2	160	532	55
Ca	44	nm	78	259	50
Ca	44	He	76	255	55
Ca	44	H_2	271	903	53
V	51	He	0.007	0.023	87
Cr	52	He	0.11	0.36	32
Cr	53	He	0.12	0.38	20
Mn	55	nm	0.03	0.10	97
Mn	55	He	0.03	0.11	98
Fe	56	He	2.1	7.1	97
Fe	56	H_2	2.6	8.6	97
Fe	57	He	2.2	7.3	96
Cu	63	nm	0.9	3.0	89
Cu	63	He	0.9	3.0	88
Cu	65	nm	0.9	2.9	91
Cu	65	He	0.9	3.0	91
Co	59	nm	0.003	0.009	89
Co	59	He	–	–	–
Ni	60	nm	0.013	0.043	90
Ni	60	He	0.020	0.066	92
Zn	66	nm	0.3	1.2	97
Zn	66	He	0.3	1.1	98
Zn	68	nm	0.4	1.2	97
Zn	68	He	0.3	1.1	98
Ga	69	nm	0.07	0.22	95
As	75	nm	0.017	0.058	82
As	75	He	0.014	0.046	–
Se	77	He	0.06	0.20	95
Se	77	H_2	0.06	0.18	97
Se	78	He	0.17	0.57	95
Se	78	H_2	0.12	0.41	98
Se	82	He	0.10	0.34	97
Se	82	H_2	0.16	0.53	92
Rb	85	nm	0.00	0.00	98
Sr	88	nm	0.11	0.36	96
Ag	107	nm	0.004	0.014	41
Cd	111	nm	0.0015	0.0050	83
Ba	137	nm	0.5	1.8	90
Pb	204	nm	0.27	0.91	–
Pb	206	nm	0.22	0.73	40
Pb	207	nm	0.23	0.76	28
Pb	208	nm	0.22	0.74	39

Table 7.2: REEs Detection limits[ng g^{-1}], quantification limits[ng g^{-1}] and repeatability of method.

Element	Mass	*LoD*	*LoQ*	Rep %
Y	89	0.003	0.011	82
La	139	0.005	0.016	86
Ce	140	0.006	0.019	88
Pr	141	0.005	0.018	87
Nd	146	0.0018	0.0059	86
Sm	147	0.0020	0.0066	86
Eu	153	0.005	0.016	95
Gd	157	0.0013	0.0044	88
Tb	159	0.004	0.012	84
Dy	163	0.0022	0.0074	85
Ho	165	0.005	0.017	88
Er	166	0.0020	0.0066	85
Tm	169	0.007	0.024	54
Yb	172	0.0020	0.0067	85
Lu	175	0.004	0.013	73

interferences). Before being used the resin must be soaked overnight in a HNO_3(1 % w/w) solution, and after removing supernatant colloidal formation, fresh acid solution have to be added. Accordin to the procedure, to perform Sr/matrix separation a pre-cleaned PP extraction cartridge has to be filled dropping the soaked Sr-resin.The resin has first to be washed with 3 mL of HNO_3 6 mol L^{-1} and then with 3 mL of sub-boiled water. If the resin is new no more washing steps are necessary, otherwise two additional washing steps have to be performed, first with 2 mL of HCl 6 mol L^{-1} and than again with subboiled water. After washing steps the resin have to be conditioned with 3 mL of HNO_3 8 mol $L^{-1}$and then can be added 1mL of sample (in $HNO_3$8 mol L^{-1})After sample loading, matrix is washed away with 10 mL of $HNO_3$8 mol L^{-1}. Strontium enriched fraction is collected rinsing the column wit sub-boiled water. All the solutions have to be added dripping 0.5 mL aliquots into the cartridge, with the exception of the sample, that is transferred drop by drop with a single 1 mL aliquot. Solutions have to be added with a slow dripping procedure in order to avoid resin resuspension and bubbles formation that would carry to imprecise results

Sr/matrix separation Evaluation

To evaluate whether the method was suitable for matrix separation of mine samples, Sr/matrix test separation was performed, using standard solutions. A preliminary screening to evaluate *Sr* and *Rb* concentration in digested samples was done using an ELAN (PerckinElmer, Ontario, Canada) ICP QMS. Based on the analysis results, six *Rb*/*Sr* standards were prepared. Mono-element Rb and Sr standards were diluted in a 8 mol L^{-1} HNO_3 solution, in order to obtain a Strontium-Rubidium ratio ranging between 0.5 and 12, as revealed in barley samples. Standards and blank solution was matrix-separated in duplicate, following the procedure previously described, and summarized in table 7.4.

To evaluate Sr recovery, different fractions were collected (Tab.7.4) and for each solution Rb

Table 7.3: Concentration calculated for SRM1567a and recovery %.

Element	Mass	Acq. mode	Certificate	Calculated	Recovery %
% by Weight					
Mg	24	nm	0.04 ± 0.002	0.039 ± 0.002	97
K	39	nm	0.133 ± 0.003	0.117 ± 0.003	88
Ca	43	nm	0.0191 ± 0.0004	0.003 ± 0.001	17
Ca	43	He	0.0191 ± 0.0004	0.003 ± 0.001	17
Ca	43	H_2	0.0191 ± 0.0004	0.012 ± 0.005	63
Ca	44	nm	0.0191 ± 0.0004	0.006 ± 0.003	29
Ca	44	He	0.0191 ± 0.0004	0.006 ± 0.002	30
Ca	44	H_2	0.0191 ± 0.0004	0.019 ± 0.008	100
$\mu g.g^{-1}$					
Na	23	nm	6.1 ± 0.8	10.4 ± 0.9	171
Al	27	He	5.7 ± 1.3	3.2 ± 0.4	57
Cd	111	nm	0.026 ± 0.002	0.024 ± 0.004	94
Fe	56	He	14.1 ± 0.5	15 ± 0.6	108
Fe	56	H_2	14.1 ± 0.5	15 ± 0.5	108
Fe	57	He	14.1 ± 0.5	15.4 ± 0.5	109
Mn	55	nm	9.4 ± 0.9	8.4 ± 0.2	89
Mn	55	He	9.4 ± 0.9	8.6 ± 0.2	91
Co	59	nm	0.006	$< LoD$	–
Co	59	He	0.006	$< LoD$	–
As	75	nm	0.006	$< LoD$	–
As	75	He	0.006	$< LoD$	–
Se	77	He	1.1 ± 0.2	1.06 ± 0.05	96
Se	77	H_2	1.1 ± 0.2	1.10 ± 0.04	100
Se	78	He	1.1 ± 0.2	1.01 ± 0.04	92
Se	78	H_2	1.1 ± 0.2	1.12 ± 0.02	102
Se	82	He	1.1 ± 0.2	1.07 ± 0.04	98
Se	82	H_2	1.1 ± 0.2	2.1 ± 0.2	187
Zn	66	nm	11.6 ± 0.4	10.7 ± 0.3	92
Zn	66	He	11.6 ± 0.4	11.0 ± 0.2	95
Zn	68	nm	11.6 ± 0.4	9.9 ± 0.3	85
Zn	68	He	11.6 ± 0.4	10.1 ± 0.2	87
Cu	63	nm	2.1 ± 0.2	1.9 ± 0.2	91
Cu	63	He	2.1 ± 0.2	2.0 ± 0.3	95
Cu	65	nm	2.1 ± 0.2	1.9 ± 0.2	90
Cu	65	He	2.1 ± 0.2	1.9 ± 0.2	91
Pb	204	nm	< 0.02	$< LoD$	–
Pb	206	nm	< 0.02	$< LoD$	–
Pb	207	nm	< 0.02	$< LoD$	–
Pb	208	nm	< 0.02	$< LoD$	–

Table 7.4: Schematic procedure for Sr/matrix separation.

Chromatography step	Reagent	Volume [mL]	Collection
Column packing	Sr spec resin	0.5	-
Cleaning 1	HNO_3 6 mol L^{-1}	3	-
Cleaning 2	Subb H_20	3	-
Conditioning	HNO_3 8 mol L^{-1}	3	-
Sample loading	In HNO_3 8 mol L^{-1}	1	-
Washing	HNO_3 8 mol L^{-1}	10	W1 - W2
Pre eluition	Subb H_20	0.5	Pre
Eluition	Subb H_20	2	Samp
Post Eluition	Subb H_20	3	Post1-2-3

and Sr concentration was screened. The recovery resulted higher than 90% (Tab.7.5), and the concentration of Rb was reduced by up to blanks levels(Tab.7.6).

Table 7.5: Sr concentration (ng g^{-1}) in screened fraction.

Stdn	*Standard*	*W*1	*W*2	*Pre*	*Sample*	*Post*1	*Post*2	*Post*3	*Tot*	*Recovery%*
Blank	0.10	0.21	0.18	0.18	0.17	0.10	0.07	0.08	−−	−−
1	126	0.13	0.13	0.29	111	1.87	0.51	0.40	114	90.5
2	142	0.14	0.13	0.22	131	3.74	1.17	0.93	137	96.4
3	149	0.13	0.11	0.22	137	3.92	0.98	1.35	143	95.9
4	320	0.14	0.14	0.38	287	8.10	2.47	2.06	300	93.8
5	441	0.14	0.12	0.19	403	13.8	4.31	2.89	424	96.1
6	621	0.16	0.14	0.32	581	15.1	5.68	3.52	605	97.5

Table 7.6: Rb concentration (ng g^{-1}) in screened fraction.

Stdn	*Standard*	*W*1	*W*2	*Pre*	*Sample*	*Post*1	*Post*2	*Post*3
Blank	0.20	0.21	0.21	0.21	0.21	0.11	0.11	0.10
1	243	18.1	0.29	0.22	0.22	0.11	0.11	0.10
2	140	24.7	0.21	0.21	0.21	0.10	0.11	0.11
3	182	13.9	0.21	0.22	0.21	0.10	0.11	0.10
4	112	9.29	0.21	0.22	0.22	0.11	0.10	0.10
5	53.9	5.07	0.21	0.21	0.21	0.10	0.10	0.10
6	54.8	6.40	0.20	0.21	0.22	0.12	0.10	0.10

Barley, malt and trashes samples purification

The preliminary screening of digested samples revealed that Rb and Sr were distributed over a wide range of concentration, but for many samples Strontium concentration was quite low in view of SF-MCICP-MS analysis. Pre-concentration via samples evaporation was performed prior to Sr/matrix separation, in order to reach an adequate Sr concentration range. 10 mL of

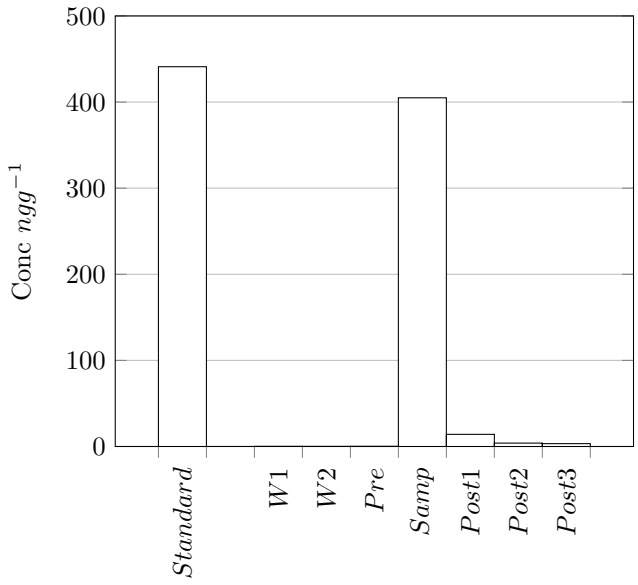


Figure 7.1: •

digested samples were transferred into PTFE vessels and leaded up to dryness with a hotplate. The temperature was kept at 95°C to ensure evaporation without sparkling. After 8-10 hours, once reached dryness, the samples were redissolved in 1 mL of HNO_3 8 mol L^{-1}. The vessels were closed with their caps, and heated for a few minutes to help samples dissolution. PTFE vessels were cleaned twice in a acid reflux cleaning system, and rinsed with milli-Q water prior to use them. Concentrated samples were purified following the separation procedure mentioned above. About 0.5 mL of resin was transferred drop by drop in a pre-cleaned PP extraction cartridge, and washed first with 1 mL of HNO_3 6 mol L^{-1} and then with SI3mLof sub-boiled water. Resin was then conditioned with 3 mL of HNO_3 8 mol L^{-1} and afterward the sample was dripped slowly into the cartridge. Sample matrix was washed away by adding 3 mL of HNO_3 8 mol L^{-1}. The resin was then rinsed with 0.5 mL of sub-boiled water, discarded before collecting in a test tube the strontium enriched fraction, obtained rinsing the column wit4 mL of sub-boiled water. All the solutions were added dripping 0.5mL aliquots into the cartridges.

After Sr/matrix separation, an aliquot of the samples was analysed by ICP-MS, for a strontium and rubidium concentration screening. It was calculated a recovery of Sr varying from 0.9 to 1.1 for al the treated samples.

7.3.2 MC-ICP-MS analysis

Strontium isotope ratio measurements were performed with a double focusing multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS)(Nu plasma HR, Nu instrument Ltd., Wrexham, U.K.). The instrument was equipped with an ESI autosampler (Elemental

scientific, Inc, Omaha, USA) and a membrane desolvating system (DSN, Nu Instrument Ltd, North Wales, UK). The instrument was settled do measure simultaneously the isotopes 84Sr, 85Rb, 86Sr, 87Sr, 88Sr, 90Zr and 91Zr. Instrumental parameters optimization was performed using a $50\,\mathrm{ng\,g^{-1}}$ standard solution NIST SRM 987. Prior to Sr isotopes analysis, samples solutions were diluted, if necessary, to reach a concentration of about $50\,\mathrm{ng\,g^{-1}}$ to obtain an optimal intensity signal when analysed. During analysis a solution of NIST SRM 987, $50\,\mathrm{ng\,g^{-1}}$ was acquired before and after every sample acquisition, to obtain a sequence SRM 987 – sample – SRM 987. Both standard and samples were spiked wit a Zr solution, to yield a final Zr concentration of $200\,\mathrm{ng\,g^{-1}}$.

7.3.3 Data analysis

The instrument gives as output a current value, for every monitored mass, already bank corrected, result of 10 consecutive reading. Data obtained was mass bias corrected using “”NICE”” method, standard sample bracketing (SSB) method and it was also applied a recent techniques (mC-SSBIN) that uses Zr internal standard to correct the temporal drift in mass bias during a measurement sequence[**Irrgeher2013**], apart from Rb correction.

7.4 Soils multielemental analysis

7.4.1 Soluble fraction extraction

Soil samples extraction was performed rearranging the procedure described in . Soils samples were dried 24 ours at 35°C in a drying oven, and then sieved with a 2-mm stainless steel sieve. About 10 g of sieved sample was transferred into a 50-mL PP centrifuge tube, and 25 g of $NH_4NO_3 1\,\mathrm{mol\,L^{-1}}$ were added. Also procedural blank were performed. Once sealed, the tubes were placed in a shaler, and the solution were stirred for 2 hours. The solution were then filtered trough a 0,45 µm PTFE filter, and stored at -20°C until analysis.

7.4.2 ICP-QMS analysis

Multielemental measurements were carried out with an inductively coupled plasma quadrupole mass spectrometer (ICP-MS) (Agilent 7500 ORS – Agilent Technologies Inc. Santa Clara, California), equipped with an octapole reaction system (ORS). The ion source was composed of a Peltier-cooled quartz spray chamber, and a quartz torch with a quartz injector tube. The instrumental performance was monitored before starting every analysis session, acquiring a multi element tuning solution, containing about $1\,\mathrm{ng\,g^{-1}}$ of 7Li, 89Y , 205Tl and 140Ce. The instrumental performance optimization was carried out adjusting torch alignment, nebulizer gas flow rate, RF power and lens voltages. An autosampler was used for solutions acquisition. In order to correct the temporal variations in signal intensity, a platinum and rhodium $10\,\mathrm{ng\,g^{-1}}$ standard

solution was added on-line. To lower as much as possible isobaric interferences the reaction cell was used , with He and H_2 as reaction gas.

Chapter 8

Conclusion and outlook

An analytical methodology was developed successfully for the determination of trace elements and rare earth elements in barley samples. The application of the developed method, supported by chemometricals analysis was capable to discriminate between barley cultivars, grown up in different Italian regions. Samples provenance was also discriminate through strontium isotopic composition that was found to be different for samples having different geographical origins. Very similar elemental profiles were found for barley and its mat and trashes. For this reason it can be supposed that provenance discrimination could be done also for barley derivate, and maybe extended to malt products. This elemental profile similarity with barley can be used for malt authentication purpose. Strontium isotopic composition of samples instead, is strongly influenced by malting process. MC-ICP-MS analysis revealed a considerable lowering in δ^{87}Sr values of malt and trashes compared with barley amount. Analysing malting process, soaking barley could be a determinant step in ^{86}Sr enrichment. To better understand this evidence, an investigation on Sr isotopic composition of the water used during this treatment could be useful. Elemental analysis of soils extracts revealed, of course, a different soluble fraction composition with respect to geographical site. Their chemical profile led to a perfect discrimination between sites. The comparison of elemental profiles of barleys with their growing soils revealed that the vegetable bioaccumulation is different for different elements. Kernels resulted to be enriched in some elements with respect of the soils. The elemental accumulation or depletion was found to be similar for the samples, no matter their provenance. To fortify the chemical relation between samples and geological background it would be recommended to investigate Sr isotopic composition of soils extracts. Furthermore acid digestion of soils samples, and the analysis of total elemental and isotopic composition would give more information. As many times mentioned in this work, multivariate statistical analysis is a powerful toll in foodstuff research. Enlarging the sample dataset, it would be possible to create robust mathematical and statistical models, capable to identify provenance of unknown samples. This can be done actually studying the elemental profiles of other barley samples grown up in the trial fields investigated [**I**]nfoAgr, and

investigating the chemical composition of malt its trashes produced from those cereals.

Eventually the analytical methodology could be optimised and applied for investigation of more complex matrix, obtaining chemical profiles of malt products, such as beer or whisky. This could yield to a widespread traceability study, involving barley from soil to consumer.

Part IV

Appendix

.1 Certificates of Analysis

ULTRA SCIENTIFIC
Analytical Solutions

Certificate of Analysis

Inorganic Custom Standard

Catalog Number:	**ICUS-1675**
Lot Number:	**G00470**
Job Number:	**J00005907**
Lot Issue Date:	**6/19/2006**
Expiration Date:	**7/31/2007**

This Certified Reference Material (CRM) was manufactured and verified in accordance with ULTRA's ISO 9001:2000 registered quality system, and the analyte concentrations were verified by our ISO 17025 accredited laboratory. The true value and uncertainty value at the 95% confidence level for each analyte, determined gravimetrically, is listed below

Analyte	True Value		Analytical Method
aluminum	10.0 ± 0.1	µg/mL	gravimetric
antimony	10.0 ± 0.1	µg/mL	gravimetric
arsenic	10.0 ± 0.1	µg/mL	gravimetric
barium	10.0 ± 0.1	µg/mL	gravimetric
beryllium	10.0 ± 0.1	µg/mL	gravimetric
bismuth	10.0 ± 0.1	µg/mL	gravimetric
cadmium	10.0 ± 0.1	µg/mL	gravimetric
cerium	10.0 ± 0.1	µg/mL	gravimetric
chromium	10.0 ± 0.1	µg/mL	gravimetric
cobalt	10.0 ± 0.1	µg/mL	gravimetric
copper	10.0 ± 0.1	µg/mL	gravimetric
gallium	10.0 ± 0.1	µg/mL	gravimetric
iron	10.0 ± 0.1	µg/mL	gravimetric
lead	10.0 ± 0.1	µg/mL	gravimetric
lithium	10.0 ± 0.1	µg/mL	gravimetric
manganese	10.0 ± 0.1	µg/mL	gravimetric
molybdenum	10.0 ± 0.1	µg/mL	gravimetric
nickel	10.0 ± 0.1	µg/mL	gravimetric
rubidium	10.0 ± 0.1	µg/mL	gravimetric
selenium	10.0 ± 0.1	µg/mL	gravimetric
silver	10.0 ± 0.1	µg/mL	gravimetric
strontium	10.0 ± 0.1	µg/mL	gravimetric
thallium	10.0 ± 0.1	µg/mL	gravimetric
vanadium	10.0 ± 0.1	µg/mL	gravimetric
zinc	10.0 ± 0.1	µg/mL	gravimetric

Matrix: 5% nitric acid in water

*Balances used in the manufacture of this standard are calibrated with weights traceable to NIST in compliance with ANSI/NCSL Z-540-1 and ISO 9001

Quality System
Quality Endorsed Company
ISO 9001
SAI Global Registered

ACCREDITED
ISO 17025
Cert. No. 0851- 01

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Edward Fitzgerald

Dr. Edward Fitzgerald,
Senior Scientist

National Institute of Standards & Technology

Certificate of Analysis

Standard Reference Material® 987

Strontium Carbonate
(Isotopic Standard)

This Standard Reference Material (SRM) is certified for use as an isotopic reference material for the calibration of mass spectrometers. The material consists of highly purified strontium carbonate of high homogeneity. A unit of SRM 987 consists of 1 g of powder.

Certified Values: The certified values for the absolute strontium isotopic abundance ratios and the atom fractions of ^{88}Sr, ^{87}Sr, ^{86}Sr and ^{84}Sr are listed in Table 1. A NIST-certified value is a value for which NIST has the highest confidence in its accuracy, in that all known or suspected sources of bias have been investigated or accounted for by NIST. A certified value is the present best estimate of the true value based on the results of analyses performed at NIST and cooperating laboratories. Value assignment categories are based on the definition of terms and modes used at NIST for chemical reference materials [1]. The uncertainties listed with the values are expanded uncertainties (95 % confidence interval) and are calculated according to the methods in the ISO and NIST Guides [2].

Table 1. Certified Values for SRM 987 Strontium Carbonate

Absolute Abundance Ratios	$^{88}Sr/^{86}Sr = 8.378\ 61 \pm 0.003\ 25$
	$^{87}Sr/^{86}Sr = 0.710\ 34 \pm 0.000\ 26$
	$^{84}Sr/^{86}Sr = 0.056\ 55 \pm 0.000\ 14$
that yield atom percents of:	$^{88}Sr = 82.584\ 5 \pm 0.006\ 6$
	$^{87}Sr = 7.001\ 5 \pm 0.002\ 6$
	$^{86}Sr = 9.856\ 6 \pm 0.003\ 4$
	$^{84}Sr = 0.557\ 4 \pm 0.001\ 5$

This material was used as the reference sample in a determination of the absolute abundance ratios and atomic weight of strontium [3]. The atomic weight of strontium calculated from the absolute abundance ratios is 87.616 81 ± 0.000 12.

Expiration of Certification: The certification of this SRM is deemed to be indefinite within the stated uncertainties. However, certification is nullified if the SRM is contaminated or otherwise altered.

Maintenance of Certified Values: NIST will monitor this SRM and, if substantive changes occur in the certified values, NIST will notify the purchaser. Registration (see attached sheet) will facilitate notification.

Stephen A. Wise, Chief
Analytical Chemistry Division

Gaithersburg, MD 20899
Certificate Issue Date: 19 June 2007
See Certificate Revision History on Last Page

Robert L. Watters, Jr., Chief
Measurement Services Division

.2 Table

Figure 1: Concentration of measured SRM [$\mathrm{mg\,kg^{-1}}$]

Eelement	Mass	SRM	*A*			*B*			*L*		
			1	2	3	1	2	3	1	2	3
Mn	55	0.17 ± 0.05	0.15	0.11	0.16	0.17	0.13	0.19	0.13	0.18	0.17
Mn∗	55	0.17 ± 0.05	0.25	0.15	0.27	0.28	0.22	0.33	0.13	0.17	0.30
Fe∗	56	1.8 ± 1.1	2.05	1.83	2.71	2.20	2.61	2.17	2.37	2.81	2.66
Fe∗	57	1.8 ± 1.1	35.50	26.46	36.15	36.30	30.48	41.03	28.65	35.81	37.33
Cu	63	0.46 ± 0.08	*nd*	*nd*	*nd*	0.45	0.36	0.50	*nd*	*nd*	*nd*
Cu∗	63	0.46 ± 0.08	*nd*	*nd*	*nd*	0.49	0.37	0.53	*nd*	*nd*	*nd*
Cu	65	0.46 ± 0.08	0.76	0.41	0.89	0.96	0.77	1.09	0.46	0.62	0.99
Cu∗	65	0.46 ± 0.08	0.47	0.33	0.47	0.44	0.39	0.50	0.36	0.55	0.46
Zn	66	28 ± 3.1	28.49	23.06	37.01	30.93	25.93	34.39	27.79	35.96	31.29
Zn∗	66	28 ± 3.1	31.61	24.25	32.32	33.53	27.17	35.75	28.95	35.27	32.53
Zn	68	28 ± 3.1	28.63	23.27	30.11	31.10	26.02	34.65	27.89	36.18	31.52
Zn∗	68	28 ± 3.1	27.42	21.05	28.01	29.03	23.63	30.83	25.18	30.58	28.06
Se∗	77	0.131 ± 0.014	0.16	0.14	0.14	0.12	0.12	0.16	0.15	0.19	0.14
Se∗	78	0.131 ± 0.014	0.10	0.14	0.13	*nd*	*nd*	*nd*	0.18	0.20	0.09
Se∗	82	0.131 ± 0.014	0.21	0.15	0.22	0.22	0.19	0.23	0.19	0.23	0.21
Sr	88	4.35 ± 0.014	5.71	3.48	5.96	4.88	5.11	7.11	3.93	5.23	6.47
Mo	95	0.29 ± 0.13	0.28	0.23	0.30	0.29	0.27	0.33	0.23	0.33	0.30
Pb	204	0.11 ± 0.05	0.13	0.09	0.10	0.10	0.10	0.17	0.07	0.10	0.16
Pb	206	0.11 ± 0.05	0.09	0.04	0.07	0.08	0.06	0.11	0.03	0.11	0.10
Pb	207	0.11 ± 0.05	0.13	0.09	0.11	0.11	0.10	0.18	0.08	0.14	0.17
Pb	208	0.11 ± 0.05	0.13	0.09	0.11	0.12	0.11	0.18	0.07	0.13	0.16
Al∗	27	0.9	0.56	0.76	0.67	0.83	0.58	0.62	0.49	0.23	0.55
Cr∗	52	0.5	*nd*	*nd*	0.03	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
Cr∗	53	0.5	*nd*	*nd*	0.03	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*
Co	59	0.003	0.02	0.01	0.02	0.02	0.01	0.02	0.01	0.02	0.01
Co∗	59	0.003	0.02	0.01	0.01	0.01	0.01	0.01	0.00	0.01	0.01
Ni	60	0.02	0.26	0.20	0.31	0.33	0.24	0.36	0.21	0.33	0.32
Ni∗	60	0.02	0.11	0.06	0.11	0.08	0.09	0.14	*nd*	0.06	0.13
As	75	0.001	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.01
As∗	75	0.001	0.01	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.00
Rb	85	16	18.87	16.97	19.46	21.26	16.07	22.81	19.60	23.05	20.75
Cd	111	0.0002	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*	*nd*

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