Laser Induced Breakdown Spectroscopy (LIBS) coupled with multivariate chemometric method for characterization and quality control of Ethiopian coffee and herbal medicines

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By Yoseph Alresawum

Addis Ababa, Ethiopia

April 2016

DECLARATION

I hereby declare that this PhD dissertation is my original work and has not been presented for a degree in any other university, and that all sources of material used for the dissertation have been duly acknowledged.

Name: Yoseph Alresawum

Signature:_____

This PhD dissertation has been submitted for examination with my approval as university advisor.

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Approved by the Examination Committee

Prof. _____, External Examiner _____

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ABSTRACT

In this researches seven Ethiopian export quality coffee, their ten mixtures, ten commonly used medicinal plants and their two mixtures were analyzed. The method of analysis we used is the LIBS technique. Atomic and ionic lines of Zn, Cu, Mg, Al, Ca, K, Mn Fe, P and Na were used for characterization of coffee and medicinal plant samples. Using the most relevant emission signals of LIBS, an attempt was made in order to discriminate the samples of coffee and their mixtures using Principal Component Analysis. We applied PCA for characterization of Ethiopian coffee, according to their geographical origin first by using forty nine atomic and ionic lines then by using all calcium and magnesium lines. We investigate several ranges and identify the use of these models on a specific range for a higher accuracy; accordingly we can able to characterize the coffee samples by using two lines of magnesium and two lines of calcium and finally we optimize our characterization by using only one line of calcium and one line of magnesium. By taking a sample at a time we have shown the capability of LIBS coupled with PCA for discrimination of coffee samples from their mixtures and this is useful for inspection of adulteration of coffee.

Nutrient elements of ten Ethiopian herbal medicines and their mixtures promoting health advancing effect and practiced in local medicine were determined using LIBS technique. The LIBS analysis revealed the presence of many nutrient elements such as copper, zinc, carbon, manganese, phosphorous, calcium, magnesium, iron, sodium and potassium. In this study we also tried to correlate the elemental compositions in the plants with their biological effects then, by using principal component analysis we clustered the herbal medicines with similar elemental compositions. Finally we demonstrated the capability of LIBS for monitoring the change in elemental compositions of the mixtures of herbal medicines.

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Supporting publication

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2. Laser Induced Breakdown Spectroscopy (LIBS) coupled with multivariate chemometric method for characterization of Ethiopian coffee for the purpose of quality control accepted for presentation on third International Congress on CoCoa Coffee and Tea held on Portugal.

List of Abbreviations

LIBS: Laser induced breakdown spectroscopy

PCA: Principal Component Analysis

FAAS: Flame atomic absorption spectrometry

FAES: Flame atomic emission spectrometry

ICP-OES: Inductively coupled plasma optical emission spectrometry

ETAAS: Electrothermal atomic absorption spectrometry

HR-CS-GFAAS: High-resolution continuum source graphite furnace atomic absorption spectrometry

FI: Flow injection

ICP-MS: Inductively coupled plasma mass spectrometry

DCP-OES: Direct current plasma optical emission spectrometry

TXRFS: Total reflection X-ray fluorescence spectrometry

EDXRF: Energy dispersive X-ray fluorescence spectrometry

ICPAES: Inductively coupled plasma-atomic emission spectrometry

AAS: Atomic absorption spectroscopy

AES: Atomic emission spectroscopy

LSC: Laser-supported combustion

LSD: Laser-supported detonation

LSR: Laser-supported radiation

LTE: Local thermodynamic equilibrium

LA-ICP: Laser ablation- Inductively coupled plasma

LA-MS: Laser ablation mass spectrometry

CF-LIBS: Calibration free laser induced breakdown spectroscopy

ICCD: Intensified Charge Coupled Device.

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INTRODUCTION

Laser induced breakdown spectroscopy (LIBS) is an emerging spectroscopic technique that operates with high-power laser pulses focused onto a small spot of the sample material. The interaction of the pulsed laser beam with the target sample produces high temperature ionized plasma, containing excited elements that radiate the characteristic emission lines of the corresponding elements. LIBS has the capability of multi-elemental analysis of any type of material present in any phase (solid, liquid and gas) with no or minimal sample preparation, real-time, in situ, remote detection, and with the capability of non-destructive determination of elemental composition .Modern analytical instruments generate large amounts of data. There are different methods for dealing with this huge quantity of information. Until recently, it was indeed impossible to fully explore a large set of data, and many potentially useful pieces of information remained unrevealed. Nowadays, the systematic use of computers makes it possible to completely process huge data collections, with a minimum loss of information. By the use of chemometric tools, it becomes possible to gain a deeper insight and a more complete interpretation of this data. The main objectives of multivariate methods in spectroscopy include data reduction, grouping and the classification of observations and the modeling of relationships that may exist between variables. The predictive aspect is also an important component of some methods of multivariate analysis. It is actually important to predict whether a new observation belongs to any pre-defined qualitative groups or else to estimate some quantitative feature such as chemical concentration.

There is an increase in the number of studies using spectroscopic approaches coupled with multivariate chemometric methods to determine the geographic origin and authenticity of commodities. Studies like these have increased public awareness and concern about the prevalence of commodities fraud in the international marketplace. Research to date covers a wide range of commodity and specialty products including wine, meat, honey, and olive oil. Studies cross a range of spatial scales e.g. comparisons among places within a country versus comparisons among countries. The analytical approaches used for site differentiation include quantification of elemental concentrations, ratios of heavy and light isotopes,

concentrations of fatty acids and quantification of rare earth elements with varying degrees of success. In looking for a commercial solution to determining the provenance of commodities, the approach used will depend upon a number of factors including the commodity type, the analytical methods available and the particular origin of commodities in question to be answered. One producer may want to distinguish their product from others produced in the same country, whilst a consumer may want to confirm the authenticity of the continent of origin.

The main objective of this thesis is to study and develop a rapid and effective laboratory based quality control methodology for characterization of Coffee and traditional medicines.

The specific objectives of this thesis are: (i) to apply PCA for characterization of Ethiopian coffee, according to their geographical origin, using the original intensities of the most relevant emission signals of LIBS, (ii) to investigate several ranges and to identify the use of these models on a specific range for a higher accuracy, (iii) to discriminate coffee samples from their mixtures for inspection of adulteration of coffee, (iv) to identify and compare the amounts of nutrient elements in the commonly used herbal medicines in Ethiopia, (v) to correlate the elemental compositions in the plants with their biological effects; (vi) to use PCA for clustering of the herbal medicines with similar elemental compositions and (vii) to show the capability of LIBS to monitor the change in elemental compositions in the mixture of herbal medicines.

The thesis organized in to two parts. Part I is about coffee and contains four chapters, chapter one deals about the basic principles and methods of LIBS, method of spectral resolution, the physics of plasma, the formation of plasma on gases, liquids and solids, the advantages of LIBS and qualitative and quantitative analysis of LIBS. Chapter two deals about coffee, it discusses about the species of coffee, the relationship between coffee quality and coffee processing, the composition of coffee, different methods of elemental analysis in coffee and about the discrimination of coffee. The third chapter is about the experimental methods used in the analysis of coffee; it describes the samples studied, the preparation of samples, the LIBS setup and the analysis. The fourth chapter deals with results and

discussions of the coffee analysis. It discusses about multivariate statistical Method PCA, the coupling of PCA with LIBS for the discrimination of mixed and pure samples of coffee and about inspection of adulteration of coffee.

Part II is about herbal medicines and contains four chapters. The fifth chapter is about the herbal medicines. It discusses about the overview of medicinal plants in Ethiopia, current status of medicinal plants in Ethiopia, quality of herbal medicines and elemental analysis of herbal medicines. The sixth chapter is about the experimental methods used in the analysis. It describes the samples, the preparation of samples, the LIBS setup and the analysis. The seventh chapter deals about the results and discussion of the analysis of herbal medicines. It discusses about the Comparison and correlation of mineral elements in herbal medicines, the clustering of the herbal medicines using PCA and about LIBS used for monitoring the change in elemental compositions of the mixture of herbal medicines. The last chapter summarizes the findings of the researches, gives conclusion and recommendations.

PART I

1

Laser Induced Breakdown Spectroscopy

1.1 History and fundamentals of LIBS

1.1.1 Introduction

In this chapter we discuss about the basic principles and methods of LIBS, method of spectral resolution, the physics of plasma, the formation of plasma on gases, liquids and solids, the advantages of LIBS and qualitative and quantitative analysis of LIBS.

Laser-induced breakdown spectroscopy (LIBS) is a method of atomic emission spectroscopy (AES) that uses laser-generated plasma as the hot vaporization, atomization, and excitation source. Because the plasma is formed by focused optical radiation, the method has many advantages over conventional AES techniques that use an adjacent physical device (e.g. electrodes, coils) to form the vaporization/excitation source. Foremost of these is the ability to interrogate samples *in situ* and remotely without any preparation. In its basic form, a LIBS measurement is carried out by forming laser induced plasma on or in the sample and then collecting and spectrally analyzing the plasma light. Qualitative and quantitative analyses are carried out by monitoring emission line positions and intensities. Although the LIBS method has been in existence for 40 years, prior to 1980, interest in it centered mainly on the basic physics of plasma formation. Since then the analytical capabilities have become more evident. A few instruments based on LIBS have been developed but have not found widespread use. Recently, however, there has been renewed interest in the method for a wide range of applications. This has mainly been the result of significant technological developments in the components (lasers, spectrographs, detectors) used in LIBS instruments as well as emerging needs to perform measurements under conditions not feasible with conventional analytical techniques. A review of LIBS literature shows that the method has a detection sensitivity for many elements that is comparable to or exceeds that characteristic of other field-deployable methods.

LIBS, an analytical method born along with the invention of the laser, has had a checkered past. First, the ablation produced by the action of the laser pulse on the sample surface was exploited as a sampling method for use with the electrode-generated spark because all materials could be ablated and the finely focused laser pulse provided micro-sampling capabilities (Moenke 1997).Subsequently, it was realized that the laser plasma generated during ablation could be used as an excitation source itself. However, with the development of high-performance laboratory-based elemental analysis methods (i.e. the inductively coupled plasma or ICP), the LIBS method was (temporarily) relegated to merely a scientific curiosity, with published literature devoted more to studying fundamental characteristics of the laser plasma than to its analytical capabilities. In recent years, however, there has been strong, renewed interest in LIBS as revealed by the number of published papers in refereed journals over the past several years. This is shown in Figure 1.1. Although there has always been a steady flow of publications dealing with LIBS, beginning in about 1995 the number per year has increased dramatically. The data in Figure 1.1 were compiled from a single database using LIBS as the keyword. Many more publications than listed here dealt with the phenomenology of the laser plasma, but LIBS, which is more closely associated with analytical applications of the plasma, was the interest here.

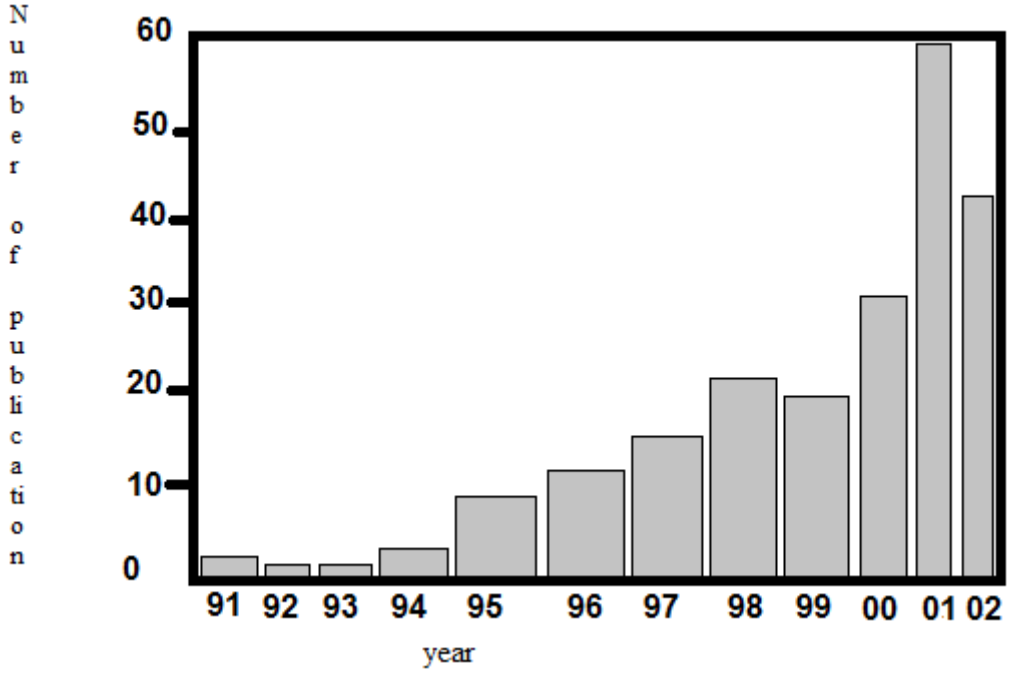


Figure 1.1: Number of LIBS-related publications. At the time of this writing, not all publications for 2002 had yet been entered into the database.

The renewed interest in LIBS can be related to several factors. First is the need for a new method of analyzing materials under conditions not possible using current analytical methods. This is driven in part by new regulations mandating that materials and operations be monitored to ensure the health and safety of workers and the public, as well as by the need for improved industrial monitoring capabilities to increase the efficiency and reduce costs of production. Second, over the past five years there have been substantial developments in reducing the size and weight while increasing the capabilities of lasers, spectrographs, and array detectors. This makes feasible the development of compact and rugged instrumentation for use in applications outside the laboratory.

1.1.3 Atomic emission spectroscopy

LIBS is one method of atomic emission spectroscopy (AES). The purpose of AES is to determine the elemental composition of a sample (solid, liquid, or gas). The analysis can range from a simple identification of the atomic constituents of the sample to a more detailed determination of relative concentrations or absolute masses. Basic steps in AES are:

- atomization/vaporization of the sample to produce free atomic species (neutrals and ions),
- excitation of the atoms,
- detection of the emitted light,
- calibration of the intensity to concentration or mass relationship,
- determination of concentrations, masses, or other information.

Examination of the emitted light provides the analysis because each element has a unique emission spectrum useful to "fingerprint" the species. Extensive compilations of emission lines exist (Reader 1980; Striganov 1968; Payling 2000). The position of the emission line(s) identifies the element(s) and, when properly calibrated, the intensity of the line(s) permits quantification. The specific procedures and instrumentation used in each step of AES are determined by the characteristics of the sample and by the

type of analysis (i.e. identification vs. quantification). It should be noted that because the first step in AES is atomization/vaporization, AES methods are generally not suitable to determine the nature of compounds in a sample. In specific cases, however, information can be obtained about molecular origins.

The beginnings of AES can be traced back to the experiments of Bunsen and Kirchhoff in which atomization and excitation were provided by a simple flame (Kirchhoff 1860; Gaydon 1957). Following this, more robust and controllable methods of excitation were developed by using electrical current to interrogate the sample. Some of the more well-known methods of vaporization and excitation include electrode arcs and sparks, the ICP, the direct coupled plasma (DCP), the microwave-induced plasma (MIP), and hollow cathode lamps (Torok 1978). These traditional sources typically require significant laboratory support facilities and some form of sample preparation prior to performing the actual analysis. In special cases, novel sampling methods have been developed for some of these sources for specific applications. Examples are an air-operated ICP providing direct analysis of particles contained in air and the introduction of particles collected on a filter into the hollow electrode of a conventional spark discharge. For various reasons, these methods saw very limited use. LIBS is an extension of the vaporization/excitation scheme to optical frequencies (Razier 1977).

1.1.4 Historic development of LIBS

The production of dielectric breakdown by optical radiation, the process generating the laser plasma used by LIBS, had to wait until the development of the laser in 1960. Prior to 1960, however, the ability to produce dielectric breakdown in gases had been known for at least 100 years. These discharges can be produced fairly easily in low-pressure gas tubes with or without electrodes, at frequencies in the range of hundreds of kilohertz to a few tens of megahertz. Examination of the spectra from these sources reveals atomic emissions characteristic of the gas composition. In subsequent years, the breakdown of gases induced by frequencies on the order of gigahertz was demonstrated at reduced pressures and atmospheric pressure using microwave range electromagnetic fields. Experiments were carried out at

reduced pressures because the breakdown threshold is minimum in rarefied gases. At atmospheric pressure, the electric field required for breakdown by static and microwave fields is of the order of tens of kilovolts per centimeter. At optical frequencies the situation requires much stronger fields on the order of 10 MV/cm. Such strong fields are not attainable using conventional optical sources, thereby requiring the development of a new light source.

In 1960, laser operation was first reported in a ruby crystal. Following this in 1963 came the development of a "giant pulse" or Q-switched laser. This laser had the capability of producing high focused power densities from a single pulse of short duration sufficient to initiate breakdown and to produce analytically useful laser plasma (also called the laser spark). This was the "birth" of the LIBS technique and in subsequent years significant milestones were made in the development of the method. Here is a list of some of the more important milestones.

- 1960 – First laser demonstrated.
- 1962 – Brech and Cross demonstrate the first useful laser-induced plasma on a surface.
- 1963 – The first analytical use, involving surfaces, hence the birth of laser-induced breakdown spectroscopy.
- 1963 – First report of laser plasma in a gas.
- 1963 – Laser micro-spectral analysis demonstrated.
- 1964 – Time-resolved laser plasma spectroscopy performed.
- 1966 – Characteristics of laser-induced air sparks studied.
- 1966 – Molten metal directly analyzed with the laser spark.
- 1970 – Continuous optical discharge reported.
- 1970 – Q-switched and non-Q-switched lasers used and results compared.
- 1972 – Steel analysis carried out with a Q-switched laser.
- 1980 – LIBS developed for analysis of hazardous aerosols.
- 1980 – LIBS used for diagnostics in the nuclear power industry.

- 1984 – Analysis of liquid samples demonstrated.
- 1989 – Metals detected in soils using the laser plasma method.
- 1992 – Portable LIBS unit for monitoring surface contaminants developed.
- 1992 – Stand-off LIBS for space applications demonstrated.
- 1993 – Underwater solid analysis via dual-pulse LIBS.
- 1995 – Demonstration of LIBS using fiber optic delivery of laser pulses.
- 1997 – Use of LIBS for pigment identification in painted artworks.
- 1998 – Subsurface soil analysis by LIBS-based cone penetrometers.
- 2000 – Demonstration of LIBS on a NASA Mars rover.

1.1.5 The LIBS method

In LIBS, the vaporizing and exciting plasma is produced by a high-power focused laser pulse. A typical LIBS set-up is shown in Figure 1.2. Pulses from a laser are focused on the sample using a lens and the plasma light is collected using a second lens or, as shown in Figure 1.2, by a fiber optic cable. The light collected by either component is transported to a frequency dispersive or selective device and then detected. Each firing of the laser produces a single LIBS measurement. Typically, however, the signals from many laser plasmas are added or averaged to increase accuracy and precision and to average out non-uniformities in sample composition. Depending on the application, time-resolution of the spark may improve the signal-to-noise ratio or discriminate against interference from continuum, line, or molecular band spectra.

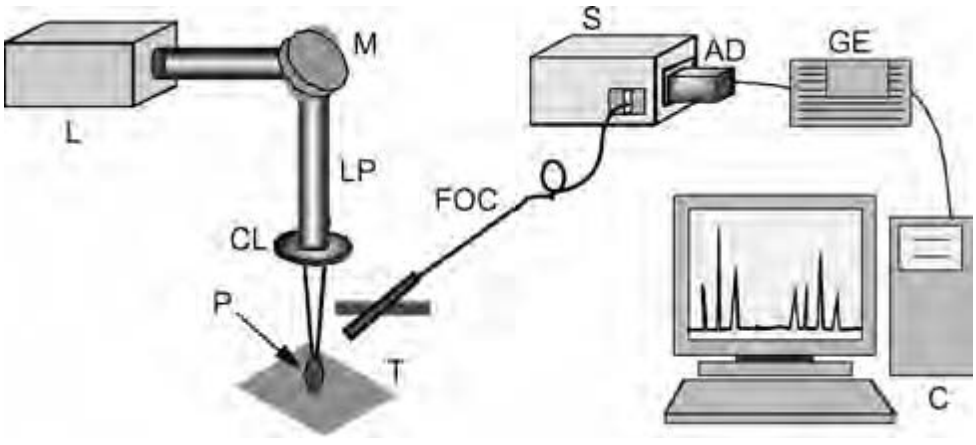


Figure 1.2: Diagram of a typical laboratory LIBS apparatus. Here: L =Nd-YAG laser; M= mirror; LP = laser pulse; CL = lens; P = plasma; T = target; FOC = fiber optic cable; S = spectrograph; AD = array detector; GE = gating electronics; C = computer.

(a) (b)

Figure 1.3: (a): the laser plasma formed on soil by a spherical lens is about 4–5 mm in height. (b): the long spark formed on a filter by a cylindrical lens is 7–8 mm in length.

Photos of laser plasmas formed on soil (by a spherical lens) and on a filter (by a cylindrical lens) are shown in Figure 1.3. To the eye, the plasma appears as a bright flash of white light emanating from the focal volume. Often the plasma formed by a spherical lens appears triangular shaped owing to formation of the initial breakdown at the focal point followed (during the laser pulse) by growth of the plasma back

towards the focusing lens. Accompanying the light is a loud snapping sound owing to the shock wave generated during optical breakdown.

Because t he l aser p lasma i s a p ulsed s ource, t he r esulting s pectrum ev olves r apidly i n t ime. T he temporal history of laser-induced plasma is illustrated schematically in Figure 1.4a. At the earliest time, the plasma light is dominated by a “white light” continuum that has little intensity variation as a function of wavelength. This light is caused by bremsstrahlung and recombination radiation from the plasma

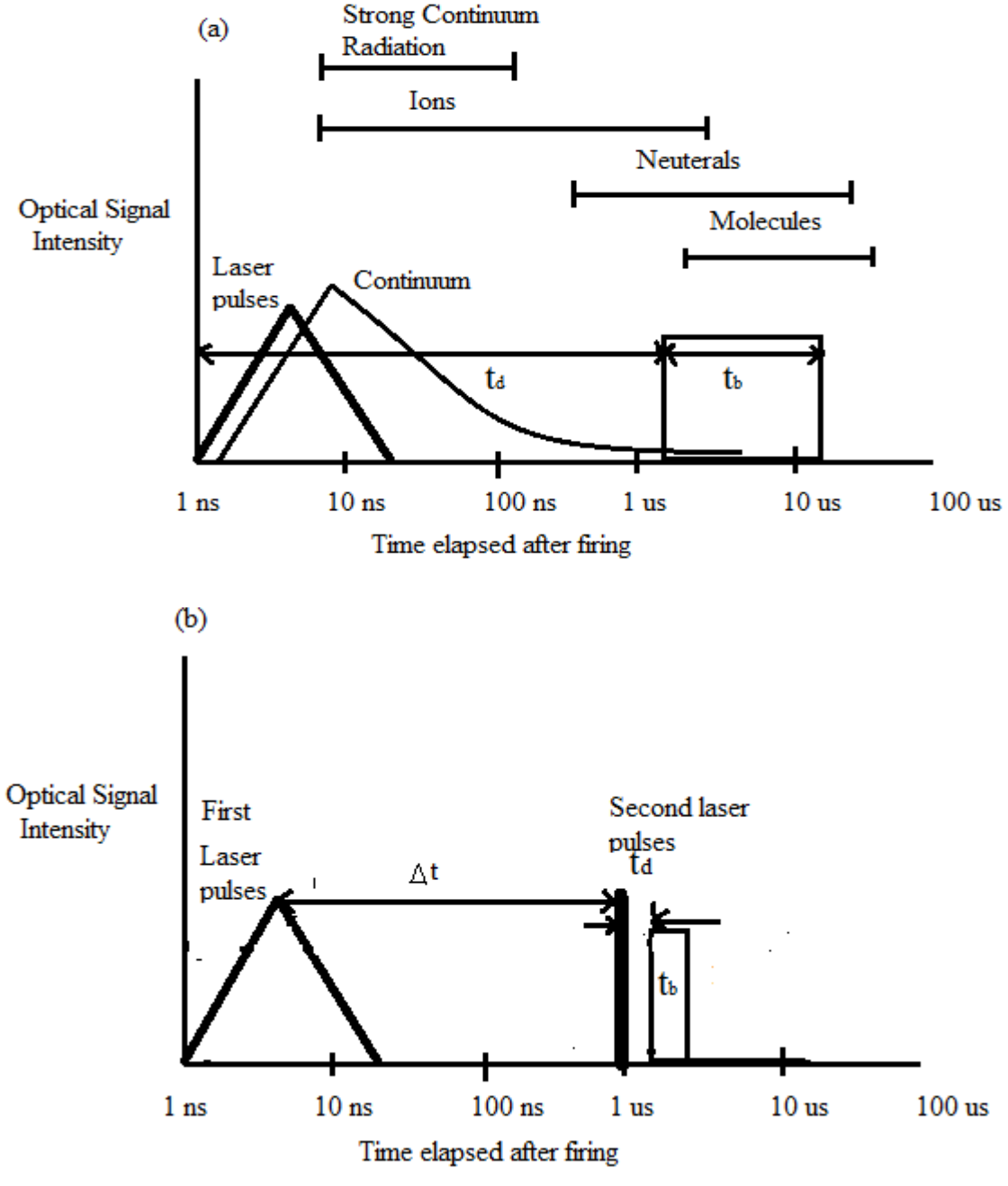


Figure 1.4: the temporal history of laser-induced plasma (a) RSS, (b) RSP

as free electrons and ions recombine in the cooling plasma. If the plasma light is integrated over the entire emission time of the plasma, this continuum light can seriously interfere with the detection of weaker emissions from minor and trace elements in the plasma. For this reason, LIBS measurements are usually carried out using time-resolved detection. In this way the strong white light at early times can be removed from the measurements by turning the detector on after this white light has significantly subsided in intensity but atomic emissions are still present. The important parameters for time-resolved detection are t_d, the time between plasma formation and the start of the observation of the plasma light, and t_b, the time period over which the light is recorded (Figure 1.4a). The majority of LIBS measurements are conducted by using the RSS (repetitive single spark) in which a series of individual laser sparks are formed on the sample at the laser repetition rate (e.g. 10 Hz). In some cases, to enhance detection capabilities, the RSP (repetitive spark pair) is used. The RSP is a series of two closely spaced sparks (e.g. typically 1–10 μs separation) used to interrogate the target at the laser repetition rate. The timing arrangement in this case is shown in Figure 1.4b. Note that t_d is measured from the second laser pulse in this case. The spark pair may be formed by two separate lasers or by a single laser.

1.1.6 Methods of spectral resolution

The basis of a LIBS measurement is the collection and analysis of an emission spectrum. The emission lines of the elements are tabulated in various sources (Reader 1980; Striganov 1968; Payling 2000). Important properties of a spectrometer are: (1) the resolution, the minimum wavelength separation at which two adjacent spectral features can be observed as two separate lines, and (2) the width of spectrum that can be observed. The specifications for these depend on the particular problem at hand. Typically a wider band of observable spectrum is needed when several elements are being monitored simultaneously.

Here are some examples of different methods for the spectral component of a LIBS system.

- Narrow-band pass (<1 nm) fixed-wavelength line filter.

- An acousto-optic tunable filter (AOTF) consisting of a crystalline material (e.g. TeO2) to which a radio-frequency wave is applied. By adjusting the frequency of the wave, the band pass wavelength of the AOTF can be varied continuously over a certain range.
- A monochromator is a spectrometer that is tuned to monitor a selected wavelength which is presented at the exit slit of the device for detection.
- A spectrograph is similar in basic configuration to a monochromator except it has an exit plane at which a continuous range of wavelengths is presented for detection using some type of array detector or a series of single-wavelength detectors positioned behind individual slits.

1.1.7 The physics of the laser plasma

A life cycle schematic for laser-induced plasma on a surface is shown in Figure 1.5. The physics of the breakdown phase was well reviewed by Weyl (Weyl 1989). Briefly, there are two steps leading to breakdown due to optical excitation (Hughes 1975). The first involves having or generating a few free electrons that serve as initial receptors of energy through three body collisions with photons and neutrals. The second is a valanche ionization in the focal region. Classically, free electrons are accelerated by the electric fields associated with the optical pulse in the period between collisions, which act to thermalize the electron energy distribution. As the electron energies grow, collisions produce ionization, other electrons, more energy absorption, and an avalanche occurs. The breakdown threshold is usually specified as the minimum irradiance needed to generate visible plasma.

Following breakdown, the plasma expands outward in all directions from the focal volume. However, the rate of expansion is greatest towards the focusing lens, because the optical energy enters the plasma from that direction. A pear- or cigar-shaped appearance results from this non-isotropic expansion. The initial rate of plasma expansion is on the order of 105 m/s. The loud sound that one hears is caused by the shockwave coming from the focal volume.

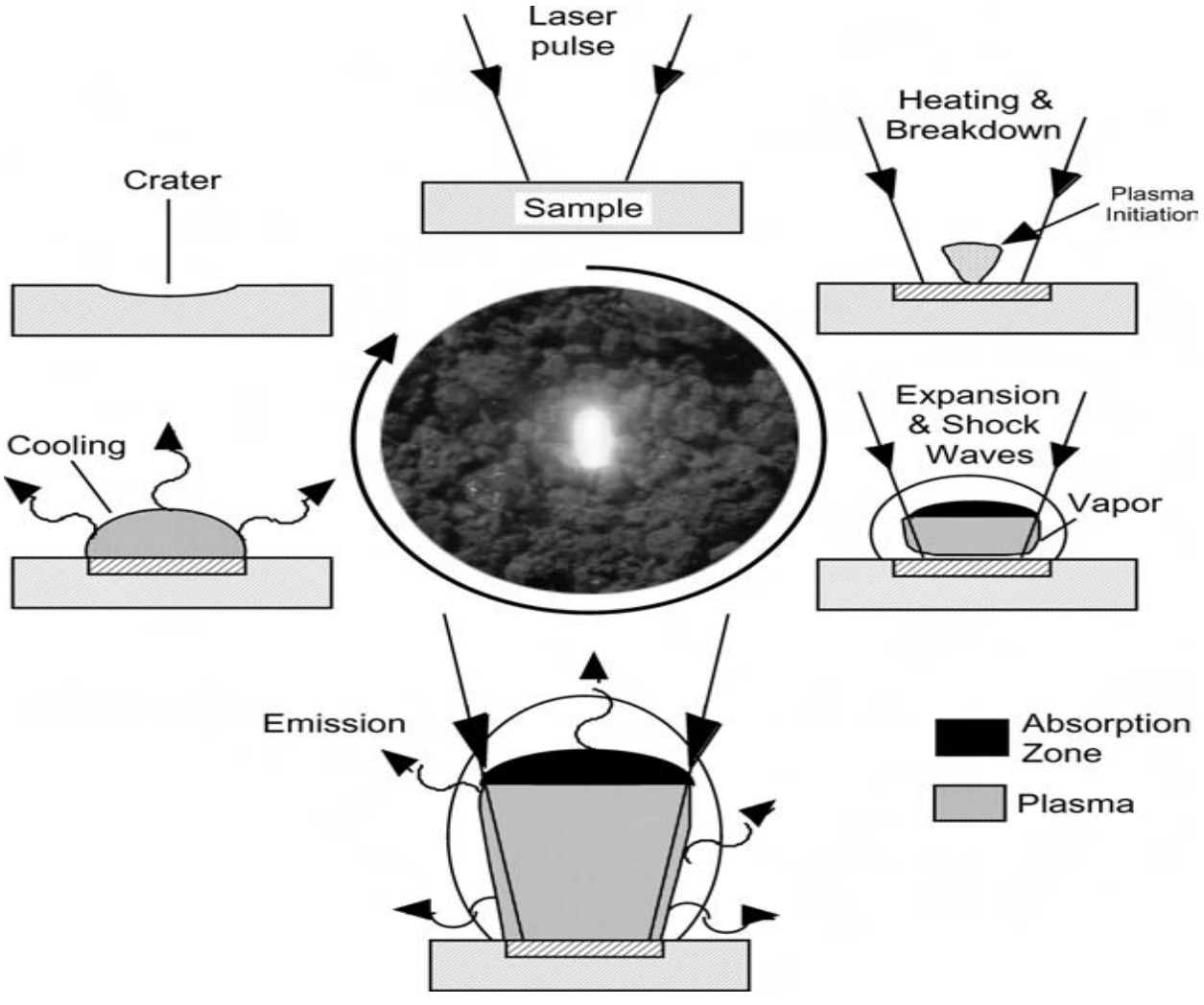


Figure 1.5: life cycle diagram showing main events in the LIBS process.

Between its initiation and decay, the plasma evolves through several transient phases, as it grows and interacts with the surroundings. These are well described, for different irradiance regimes, by Root (Root 1989). The three models for propagation and expansion are the laser-supported combustion (LSC), laser-supported detonation (LSD), and laser-supported radiation (LSR) waves. They differ in their predictions of the opacity and energy transfer properties of the plasma to the ambient atmosphere. At the low irradiances used in LIBS experiments, the models that most closely match experiment are LSC and LSD. In these, the plasma is at relatively low temperature and density. The plasma and the boundary with the ambient atmosphere are transmissive enough to allow the incoming laser radiation to penetrate, at least for laser wavelengths shorter than that of the CO_2 laser (10.6 μm).

Throughout the expansion phase the plasma emits useful emission signals. It cools and decays as its constituents give up their energies in a variety of ways. The ions and electrons recombine to form neutrals, and some of those recombine to form molecules. Energy escapes through radiation and conduction

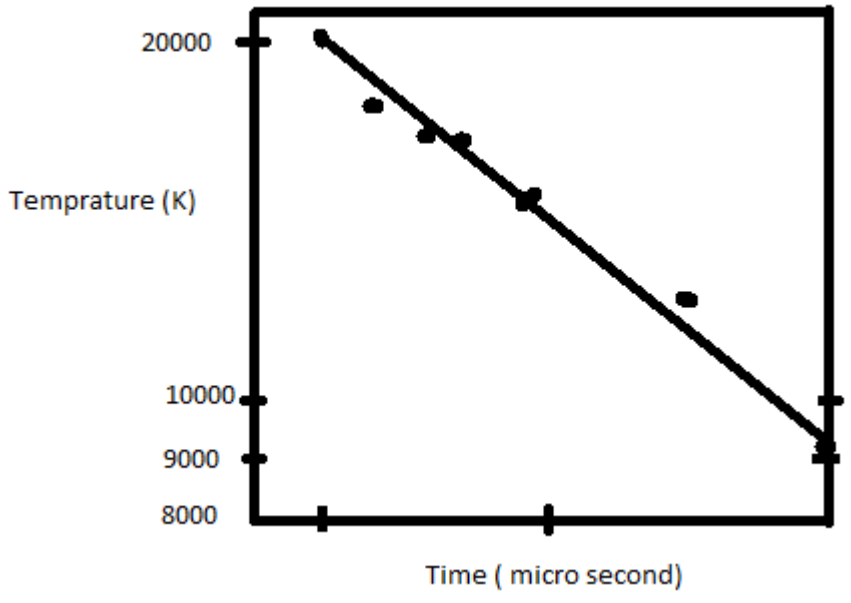


Figure 1.6: Air plasma temperature as a function of time after plasma formation. Data abstracted from reference using Saha and Boltzmann data from carbon and beryllium lines.

Typical plasma temperatures up to tens of thousands of degrees (several electron-volts) are achieved shortly after plasma initiation, for laser pulse energies of 10–100 mJ, and lens focal lengths of 5–20 cm, giving irradiances in the range of 10^9 to 10^{11} W/cm^2. Figure 1.7 presents composite measurements made of plasma temperatures in air as a function of time (Griem 1984). The temporal dependence leads to the experimental strategy of using a time delay for LIBS measurements. Because the early spectrum contains a bremsstrahlung and recombination continuum that decays quickly, the atomic signals (both ion and neutral) are often not sampled until after a microsecond or more into the plasma history (Figure 1.4). At that time the signal to background improves dramatically, and the atomic emission lines become much sharper.

At early times, spectral-line broadening is dominated by the Stark effect due to the high initial density of free electrons and ions. Line widths are dramatically dependent on the species, being greatest for the Hα line of hydrogen at 656 nm. The theory of Stark effect for atoms, including the dependence on the energy levels of the particular atom, is well developed (Simeonsson 1994). Figure 1.7 shows measurements of the electron density as a function of time for plasmas in air at different delay times. As the plasma evolves in the post-laser pulse regime, recombination occurs, the electron density decreases, and pressure broadening (Stark effect due to near collisions with neutrals) is often the main cause of the line width. The pressure and nature of the ambient gas influence the absolute line intensities, the line widths, and in some cases relative line intensities due to near-resonant collisions. Some experiments have shown that an argon atmosphere enhances excitation, while a helium or oxygen atmosphere suppresses excitation.

Laser wavelength may have an effect on breakdown threshold as discussed in a systematic study by Simeonsson and Miziolek (Radziemski 1985). They studied plasmas formed in CO and CO_2 gases with excitation by ArF at 193nm and the four common Nd:YAG wavelengths: 266, 355, 532, 1064 nm. The principal effect observed was the reduction of the breakdown threshold by an order of magnitude at 193 nm owing to fortuitous coincidences of two-photon excitations and dissociations. Otherwise the results showed no significant trends. Weyl (Weyl 1989)reviews and presents a theory that bears on the wavelength dependence. Within the past decade, there have been studies of resonance effects, where the plasma-forming laser is tuned to a strong line in an element in the sample.

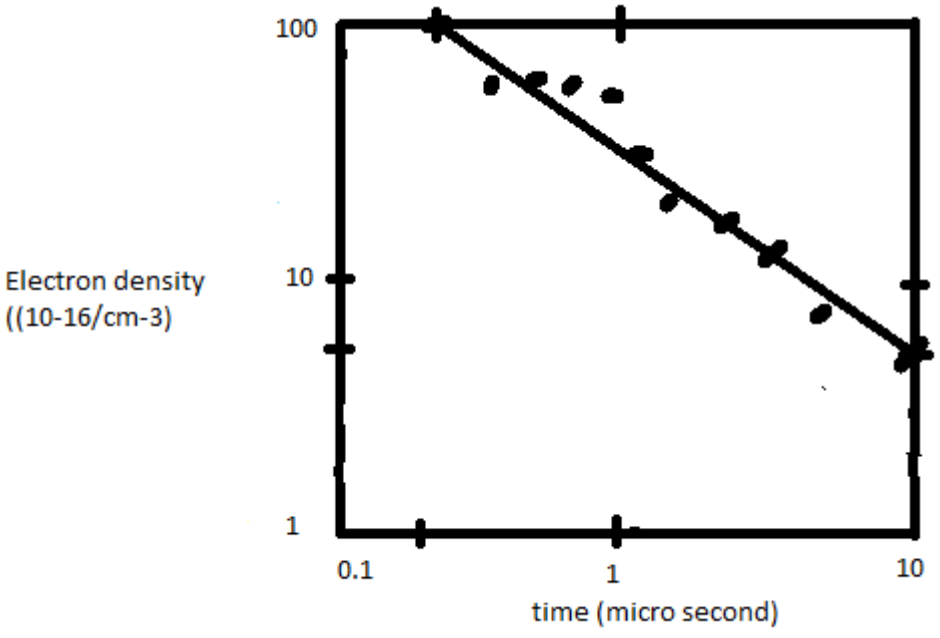


Figure 1.7: Electron density in the air plasma as a function of time after plasma formation. Data abstracted from reference using Stark widths of F, Ar, N, and Cl lines.

As is well known, plasma temperatures can be determined in a variety of ways, including spectroscopic and probe methods. There are electron, excitation, and ionization temperatures, to name just a few. Each is determined based on different diagnostics and measurements, and they may or may not agree. The situation is complicated by the transient nature of pulsed plasmas. Generally, pulsed plasmas do not start out in equilibrium, but evolve to that state. Often electrons start out at a much higher kinetic temperature, and eventually equilibrate with the heavier atoms and ions through collisions (Mazhukin 1994). Physics tells us that momentum transfer is small in collisions between bodies of very different masses; hence the time scale for equilibration between electrons and atoms can be quite long.

Through the 1980s much of the effort in analyzing LIBS plasmas was spent on measuring temperature and electron densities. Starting from the early 1990s researchers have attempted to model the plasma in more detail by using hydrodynamic codes and various plasma models. Outcomes include species densities as functions of time and other parameters, an understanding of excitation mechanisms in

various regimes, and a clearer understanding of if LTE (local thermodynamic equilibrium) may or may not apply.

1.1.8 Forming the LIBS plasma in a gas, liquid, and on solids

Initial LIBS work concentrated on the analysis of solids (e.g. metals, geological samples) but, as the unique sampling capabilities of the laser spark came to be realized, the technique was extended to a variety of other samples. Today LIBS is used to analyze gases, liquids, particles entrained in gases or liquids, and particles or coatings on solids.

Gases

In gases, less energy is used in the atomization process, so more energy is left for excitation. In general, the greater the irradiance, the greater is the initial ratio of ions to neutral atoms. Breakdown thresholds are slightly higher in gases than on surfaces, unless the gas contains particulate matter. The plasma volume depends on the energy per pulse and the laser wavelength. For nominal pulse energies of 200 mJ, the plasma length would be largest for high-energy CO_2 laser pulses (5–8 mm in length), and smallest for 266 nm Nd:YAG pulses (1–5 mm). Molecular gases can be completely dissociated by the plasma and the composition of neat molecular gases can be inferred. Typical plasma temperatures of 20 000 K or more are observed early in the plasma lifetime.

Liquids

Liquids can be analyzed by forming the laser plasma on the liquid surface (Wachter 1987) or on drops of the liquid (Archontaki 1988). If the liquid is transparent at the laser wavelength, plasma can be formed in the bulk liquid below the surface (Cremers 1984). Compared with LIBS analysis in air, the plasma formed in the bulk liquid decays more rapidly, emission lines appear broader, and the temperature is lower, typically starting at no more than 7000–12 000 K. Detection limits for selected elements in aqueous media can be increased by using a double-pulse RSP technique in which two sequential laser pulses, separated in time, typically by microseconds, interrogate the same volume of the sample. The first pulse produces a vapor cavity that is then interrogated by the second pulse, replicating an analysis

that would be carried out in air. This method works for both the bulk liquid and liquid drops. Adding an absorber to the liquid may enhance plasma formation.

Particles

Particles entrained in liquids (hydrosols) or gases (aerosols) are of great interest for environmental monitoring. Two basic strategies for obtaining information on them are (1) direct monitoring in the ambient medium and (2) capture on filters with subsequent interrogation of the filter surface by the laser spark (Figure 3.3). Some of the spark energy is used to ablate or vaporize the aerosol. Typically there is enough energy remaining to do the excitation, obtaining strong spectra and reasonable detection limits. Incomplete vaporization of the particles, a real possibility, however, can complicate quantification. There is continuing interest in determining particle compositions and loading, often for environmental applications.

Solids

Breakdown on surfaces and ablation are complex phenomena. Depending on the pressure above the surface, breakdown can be initiated by multi-photon ionization (low pressure), or inverse bremsstrahlung (high pressure), both followed by avalanche ionization. Breakdown thresholds are two to four orders of magnitude lower than in the case of gas targets. For low pressures above the surface, higher ion stages are reached for the same incident intensities. Several studies have looked at the evolution and build up of the plasma as a function of time, position, and incident laser wavelength. Regarding wavelength, long wavelengths like that from the 10.6 μm CO_2 laser have a different effect than a short wavelength such as 248 nm from a KrF laser. The long wavelength is absorbed to a much greater extent in the plasma above the surface because absorption varies as the square of the wavelength (λ^2). This effectively shields the surface from absorption of the trailing edge of the laser pulse. At short wavelengths, a higher percentage of the laser energy impacts the surface.

1.1.9 Advantages of LIBS method

LIBS, like other methods of AES, has the following advantages compared with some non- AES-based methods of elemental analysis are ability to detect all elements and simultaneous multi-element detection capability.

In addition, because the laser spark uses focused optical radiation rather than a physical device such as a pair of electrodes to form the plasma, LIBS has many distinct advantages compared with conventional AES-based analytical methods. These are:simplicity, rapid or real-time analysis, no sample preparation, allows *in situ* analysis requiring only optical access to the sample, ability to sample gases, liquids, and solids equally well, good sensitivity for some elements (e.g. Cl, F) difficult to monitor with conventional AES methods, adaptability to a variety of different measurement scenarios and robust plasma that can be formed under conditions not possible with conventional plasmas.

1.2 Quantitative analysis

The aim of this section is to provide basic information on the use of the technique of laser induced breakdown spectroscopy (LIBS) for quantitative analysis. It begins with a discussion of the theoretical assumptions on the state of the plasma that must be made in order to ensure reliability of the analysis. A review is then presented of some of the methods developed to extract quantitative information from experimental LIBS data.

In 1997, Castle *et al*. stated that at the time only a limited number of studies had reported on the use of LIBS as a quantitative technique (Castle 1997). This paucity of results was attributed to the inadequate level of the analytical figures of merit (accuracy, precision and detection limits) attainable by this technique in comparison with other well-established techniques. Since then, however, many papers have appeared in the literature reporting on the use of the LIBS technique for quantitative analysis. In fact, owing to the peculiar advantages of LIBS, including short measurement times, the ability to use samples without any pre-treatment and the capability for simultaneous multi-element detection, many researchers

have focused their efforts on developing new methods for reliable LIBS-based quantitative analysis. Undoubtedly, in some particular situations (screening, *in situ* measurement, process monitoring, hostile environments, etc.) LIBS may be the technique of choice. Thus, the main research efforts have been aimed at exploiting the technique's potential and minimizing its drawbacks.

Most of the drawbacks of LIBS are, however, side effects of its intrinsic advantages. For example, because of the small size of the focused laser beam and the small sample mass vaporized by the spark, the accuracy is heavily dependent on the homogeneity of the sample. In addition, the lack of sample preparation means that small amounts of surface contaminants may affect the analysis and, at the same time, reproducibility of the laser spark may be reduced by changes in surface composition. Also, the pulsed operation of the spark yields a lower integrated emission signal and less reproducible sample excitation than a continuous excitation source such as an inductively coupled plasma (Cremers 1987). However, most of these problems can be alleviated through proper choice of the experimental conditions.

Here we review the approaches used for extracting quantitative data from LIBS. Before presenting a brief discussion of the methods, however, it is worthwhile to examine the fundamental spectrochemical hypotheses underlying the laser-induced plasma state. Only those issues strictly related to achieving precise quantitative analysis via LIBS will be covered. In keeping with the literature, most of the discussion refers to the analysis of solid samples. However, some interesting results are achieved in the field of liquid or aerosol LIBS analysis

1.2.1 The characteristics of laser-induced plasma and their influence on quantitative LIBS analysis

Laser-induced plasma emission consists of atomic and ionic spectral lines characteristic of the constituent species, superimposed on a broad-band continuum that is the result of electron–ion recombination and free–free interactions. Identification of the spectral lines and measurement of their intensities provide qualitative and quantitative information, respectively. Quantitative analysis is not a trivial task: the spectral emission intensity in the plasma is determined not only by the concentration of

the element in the sample, but also by the properties of the plasma itself, which in turn depend on factors such as the characteristics of the excitation source (energy, power density, and wavelength) also on the sample and the surrounding gas. Furthermore, the laser ablation process (a term which includes the processes of evaporation, ejection of atoms, ions, molecular species and fragments; hydrodynamic expulsion; shock waves; plasma initiation and expansion; plasma–solid interactions; etc.) influences the amount and composition of the ablated mass and must be understood and controlled in order to achieve accurate and sensitive quantitative analysis (Russo 1995).

The complexity of the phenomena involved can be appreciated by resorting to a simple derivation of the dependence of the LIBS signal upon the various processes leading from the (solid) sample to the measured signal photons emitted from the (gas phase) atoms and ions excited in the plasma volume. The fundamental parameters governing the overall process can then be explicitly factored out. The signal, S (counts), due to emission of a particular atomic or ionic line of an element is given by the product of the excited state number density, n_u (cm^{-3}), the spontaneous transition probability of the transition chosen, A_{ul} (photons s^{-1}), and the *detection function*, f_{det} (cm^3 counts photon^{-1} s). This function can be defined here as the product of the excitation volume V_{exc} (cm^3), seen by the detector, and of an overall detection efficiency, η_{det} (counts photon^{-1} s),which includes such "trivial" parameters as optical transmission, detector gain (counts photon^{-1}), integration time (s) and a calibration function, f_{cal} (no units), which describes the plasma characteristics in terms of optical depth, i.e. the probability that photons emitted in the plasma center escape through the remaining plasma volume and reach the detector. Therefore, f_{cal} includes self-absorption (and self-reversal) effects. In symbols,

$$S = n_u A_{ul} f_{det} = n_u A_{ul} V_{exc} f_{cal} \eta_{det} \qquad 1.1$$

The total number of excited atoms (ions) in the emitting state ($\equiv n_u V_{exc}$) must be related to the total number of atoms (ions) created by the laser and present in the gas phase in the plasma volume, $(N_T)_g$, multiplied by an *excitation/ionization function*, f_{exc} (no units), which gives the probability of occupation of that particular atom/ion emitting level among all other possible levels. Therefore,

$$S = (N_T)_g \, f_{exc} A_{ul} f_{cal} \, \eta_{det} \quad 1.2$$

The total number of atoms (ions) in the excitation volume, $(N_T)_g$, must be related to the total number of atoms in the sample through an *ablation/vaporization function*, f_{abl}, which describes the mechanism by which a certain fraction of the solid material is ablated and carried in the vapor phase by the developing plasma plume. Accordingly,

$$S = (N_T)_s f_{abl} \, f_{exc} A_{ul} f_{cal} \, \eta_{det} \quad 1.3$$

The product $(N_T)_s f_{abl}$ can also be factored out and represented by the product of three factors: (i) the ratio $(m_s N_A / M_s)$, where m_s (g) is the ablated sample mass, N_A is theAvogadro number (atoms/mole) and M_s is the atomic weight (g/mole) of the element; (ii) the weight fraction of the element in the sample, χ_s; and (iii) a stoichiometric factor, f_{st}, which can be defined as the deviation of the elemental composition of the sample in the gas phase as compared with that in the solid phase. In other words, if only two elements (*i* and *j*) are considered, f_{st} can be defined by

$$f_{st} = \frac{(N_i/N_j)_g}{(N_i/N_j)_s} \quad 1.4$$

Combining all the above expressions (2.1–2.4) we obtain

$$S = A_{ul}(m_s(N_A/M_s)\chi_s f_{st}) \, f_{exc}(f_{cal} \, \eta_{det}) \quad 1.5$$

Or

$$S = A_{ul} f_{int} \, f_{exc} \, f_{det} \quad 1.6$$

It can then be concluded that the signal is influenced by three interrelated functions, describing the initial interaction between the sample and the laser, f_{int} (leading to ablation/ vaporization of solid material), the excitation/ionization mechanism leading to atomic (ionic) emission, f_{exc}, and the characterization of the radiation environment, f_{det} (thin or thick plasmas). These functions can now be discussed separately and their relevance to analytical LIBS assessed. In this way, it should be possible to point out the problems and pitfalls of the technique, to review the attention and solutions given to each of these functions in the literature and to indicate the areas where further work is necessary.

In an attempt to simplify a very complex phenomenon, most of the methods developed for quantitative LIBS analysis assume, either implicitly or explicitly, that:

- the composition of the plasma volume under observation is representative of the sample composition (stoichiometric ablation),
- the plasma volume under observation is in Local Thermodynamic Equilibrium (LTE), and
- the spectral lines measured are optically thin.

In the following, it will become clear that even when such hypotheses seem to be superfluous (for example, when quantitative LIBS analysis is performed using the calibration curves obtained from known reference samples), the reproducibility of the quantitative results is, in most cases, assured only when these basic assumptions are satisfied.

1.2.2 Stoichiometric ablation: the interaction function

Understandably, the problems of ablation efficiency and stoichiometric material removal have been addressed by numerous studies and authors. Only a few pertinent references will be included in this overview. The hypothesis of stoichiometric ablation forms the very basis of the LIBS method (as well as other techniques which rely on laser ablation for the sampling stage: LA-ICP, LA-MS, etc.). Indeed, in 1991 Chan and Russo (Chan 1991) demonstrated that laser ablation is stoichiometric when the power density on the target exceeds $10^9 W cm^{-2}$, a value that is commonly reached in LIBS measurements. In several subsequent papers, Russo and co-workers (Russo 1995; Chan 1991; Mao 1996) provided further insight into the phenomenon as well as a more detailed explanation of the processes which lead to the establishment of stoichiometric ablation. According to their studies, laser–material interactions can be described by using two different models: vaporization or ablation. A vaporization process is generally involved at power densities $\leq 10^6 W cm^{-2}$, typically corresponding to microsecond or longer laser pulses. Phonon relaxation rates are on the order of 0.1 ps; therefore, the absorbed optical energy is rapidly converted into heat. Heat dissipation and vaporization are rapid in comparison with the laser pulse duration. Differential vaporization is possible in such cases because the higher vapor-pressure

elements will be enriched in the vapor phase with respect to the original solid sample. At higher power densities, $\geq 10^9 W cm^{-2}$, corresponding to nanosecond and shorter laser pulses, an explosion occurs. The vaporization temperature of the surface is exceeded within a fraction of the laser pulse duration. However, before the surface layer can vaporize, the underlying material reaches its vaporization temperature, causing the surface to explode. The rapidly heated material has the same composition as the solid, and the process results in stoichiometric ablation. Recently, Russo *et al*. (Russo 2002) discussed five distinct regimes in the laser/solid interaction process with four transition points or thresholds. Nano-, pico and femto-second laser pulses were considered. The processes described by Russo and co-workers offer an important guideline to the researcher preparing a LIBS experiment. In any event, it should be kept in mind that this model cannot cover all possible experimental situations, so that in practical LIBS measurement, the occurrence of stoichiometric ablation should be checked a posteriori for the specific class of materials under analysis.

For example, a case of non-stoichiometric ablation has been reported by Mao *et al*. (Mao 1998), in which laser ablation of a brass sample was performed using different lasers with various wavelengths and pulse durations. For a 30 ns pulse-duration excimer laser at lower power density, the interaction was dominated by thermal vaporization, and the vapor was enriched in zinc (the latent heat of vaporization for zinc is lower than that for copper by a factor of about 3). Using a picosecond Nd:YAG, on the contrary, the resulting vapor was enriched in copper at lower power density. In order to explain such findings, a non-thermal mechanism was proposed, which involves an interaction between space charges and ionized species at the sample surface. The fast photoelectrons generated by a picosecond pulse can, in fact, produce space charges. In this case, at lower power density the ionization of copper (ionization potential = 7.72 eV) could be favored over that of zinc (ionization potential 9.29 eV). However, for both types of laser, increasing the laser power density eventually leads to stoichiometric ablation. Nouvellon *et al*. (Nouvellon 1999), on the other hand, using a KrF laser at 248 nm, could not verify stoichiometric ablation in the case of copper in brass samples. Another condition affecting stoichiometric ablation is the

formation of a deep crater by repetitive irradiation of the same surface position. Borisov *et al.* (Borisov 2000) investigated the dependence of the Pb/U line emission intensity ratio on the number of laser pulses focused on the same spot in a NIST glass standard. This particular element pair was chosen for the large difference between the melting points of their corresponding oxides. For power density values above a certain threshold (previously determined by the same group), the ablation was stoichiometric as long as the crater was shallow. However, when the crater aspect ratio (depth/diameter) exceeded a value of about 6, the composition of the ablated material diverged from stoichiometric, becoming enriched in lead. Two interpretations of this behavior are possible: the first attributes the deviation from stoichiometry to the effect of reduction of the actual power density within the crater, owing to geometric factors, to values below the threshold for stoichiometric ablation; the second, instead, points to the shielding and thermal effects of the plasma generated inside the crater. In any event, the authors conclude that good stoichiometric ablation can be achieved when experimental conditions are carefully selected.

Investigation of an issue analogous to stoichiometric ablation in solids has been undertaken by Dudragne *et al.* (Dudragne 1998) for the detection of fluorine, chlorine and carbon compounds in air. They found that the slope of the calibration curves of the carbon-line versus the concentration of the compounds in air (expressed as volume/volume ratio) is proportional to the number of carbon atoms in the molecule. The same has been observed for chlorine and fluorine compounds, demonstrating that the gaseous molecules are completely dissociated in the plasma (similar conclusions have been reached by Cremers and Radziemski (Cremers 1983)). This finding enabled the authors to calculate a calibration curve normalized to one atom per molecule for each element considered and to determine the stoichiometric ratio between fluorine, chlorine and carbon for unknown compounds. Similarly, Winefordner and co-workers (Tran 2001) have demonstrated a stoichiometric relationship for the determination of C:H:O:N ratios in solid organic compounds. As a final remark, it should be noted that, because only a small amount of material is sampled and analyzed in LIBS, the accuracy and precision of the measurement are

heavily dependent on the homogeneity of the sample. A particular case is represented by the analysis of aerosols, in which deviation from stoichiometry may be the result of incomplete vaporization of particles, because some elements are segregated on the grain surface or interior. For instance, in a study based on Be particles, Cremers and Radziemski (Cremers 1985) suggested the existence of an upper limit to the mass that can be vaporized by single laser pulses and observed that under the specific experimental conditions used, particles greater than 10 μm in diameter were under sampled. This value has been often quoted in the literature as the upper limit for complete particle vaporization in LIBS but it is certainly very dependent upon laser fluence and particle composition and may vary widely. Recent measurements by Carranza and Hahn (Carranza 2002) on SiO_2 particles have yielded an upper size limit of about 3 μm.

In general, the use of shorter laser pulses should be beneficial with respect to fractionation problems (Russo 2002). Also, the ablation efficiency (defined here as the ratio of the ablated matter volume to the laser pulse energy) was found to be better when a femtosecond laser was used (Semerok 1999).

1.3 The excitation/ionization function

1.3.1 Definition of thermodynamic equilibrium in laser-induced plasmas

In laser-induced plasmas, the duration of the plume emission is long compared with both the radiative lifetimes of the emitting species and the laser pulse length. Therefore, plasma emission is not a direct consequence of the photo-excitation mechanism. Rather, a longer lived secondary process, such as impact excitation by thermal electrons, has been invoked to explain the phenomenon (Cheung 1997). Owing to the nature of the particles making up the plasma, we would expect the kinetic, excitation, ionization, and radiative energies to contribute to the description of the system state. The distributions corresponding to the above-mentioned energy forms are described respectively by the Maxwell, Boltzmann, Saha and Planck functions. The equilibrium distribution of energy among the different states of the assembly of particles is determined by the temperature, *T*, defined for each particular form of energy. It may happen that an equilibrium distribution exists for one of these forms of energy, but not

for another. Complete thermodynamic equilibrium would exist when all forms of energy distribution are described by the same temperature. Under such conditions, the principle of detailed balance must hold. In practice, this situation cannot be fully realized, and some approximations must be adopted to describe the plasma state. The form of energy that is most often decoupled from the others is radiation energy, since radiative equilibrium requires the plasma to be optically thick at all frequencies. However, typical LIBS plasmas, in which electron collision is the rate-determining mechanism, can be described by a state known as Local Thermodynamic Equilibrium (LTE). In this state, the collision processes must be much more important than the radiative ones, so that the non-equilibrium part of radiative energy can be neglected, while for every point it is still possible to find a temperature parameter that satisfies the Boltzmann, Saha and Maxwell distributions. Thus, the plasma electronic excitation temperature, T, and the electron density,n_e which can be derived from the plasma emission data, can be used to describe the plasma characteristics. .

In referring to the plasma constituents, we need to distinguish between chemical elements (whose concentration we wish to measure in the sample) and species corresponding to different ionization stages of the same element present in the plasma. By convention, spectroscopic notation indicates neutral and single ionized species of the element Pb, for instance, as Pb(I) and Pb(II), respectively. It is widely recognized that, in typical LIBS plasma and within the typical measurement time window, only neutral atoms and singly charged ions are present to a significant degree. Therefore, in the following, only neutral and singly ionized particles will be considered. In any case, all the relations can, if necessary, be easily generalized to include higher ionization states. Under LTE conditions, the population of the excited levels for each species follows a Boltzmann distribution (see the f_{exc} in equation (1.2)):

$$n_i^s = \frac{g_i}{Z^s(T)} n^s e^{-E_i/KT} \qquad 1.7$$

where n_i^s indicates the population density of the excited level i of species s, g_i and E_i are the

statistical weight and the excitation energy of the level, respectively, n^s is the total number density of the species s in the plasma, K is the Boltzmann constant and $Z^s(T)$ is the internal partition function of the species at temperature T:

$$Z^s(T) = \sum_i g_i e^{-E_i/KT} \quad 1.8$$

Here, and in what follows, the ground state of the atom or ion corresponds to zero energy. Owing to the crowding of energy levels toward the ionization limit, calculation of the partition function should, in principle, include infinite terms, especially at high plasma temperatures, causing the sum to diverge. However, the sum extends to infinity only if the possible principal quantum number and, hence, the atomic radius extend to infinity. In the plasma environment, however, because of screening by the other charged particles, the electron is attracted by the nucleus until it is within a finited istance (corresponding to the radius of the Debye sphere). This is equivalent to reducing the effective ionization potential E_{ion} for each species in the plasma by a factor ΔE_{ion} . This same factor defines a cut-off limit to the sum of the partition function, thereby removing the problem of divergence (Thorne 1974).

The condition that atomic and ionic states should be populated and depopulated predominantly by electron collisions, rather than by radiation, requires an electron density which is sufficient to ensure a high collision rate. The corresponding lower limit of electron density n_e is given (cm−3) by the McWhirter criterion:

$$n_e \geq 1.6 \times 10^{12} T^{1/2} (\Delta E)^3 \quad 1.9$$

where ΔE (eV) is the highest energy transition for which the condition holds, and T (K) is the plasma temperature. This criterion is a necessary, though insufficient, condition for LTE, and is typically fulfilled during the first stages of plasma lifetime. It is, however, difficult to satisfy for the low-lying states, where ΔE is large. However, for any n_e, it is possible to find high excitation levels where the

states are close enough for equation (1.9) to hold. In this case, the plasma is said to be in partial LTE (Thorne 1974).

As already mentioned, LTE plasmas can be characterized by a single temperature that describes the distribution of species in energy levels, the population of ionization stages or the kinetic energy of electrons and heavier particles. Consequently, the excitation temperature which controls the population of the atomic (and ionic) energy levels should be the same as the ionization temperature, which determines the distribution of atoms of the same element in the different ionization stages. This latter distribution is described by the Saha equation, which in the case of the neutral and singly ionized species of the same element can be written as

$$n_e \frac{n^{II}}{n^I} = \frac{(2\pi m_e KT)^{3/2}}{h^3} \frac{2Z^{II}(T)}{Z^I(T)} e^{-E_{ion}/KT} \qquad 1.10$$

where n_e is the plasma electron density, n^I and n^{II} are the number densities of the neutral atomic species and the single ionized species, respectively, E_{ion} is the ionization potential of the neutral species in its ground state, m_e is the electron mass, and h is Planck's constant. It is worth mentioning that in accurate calculations, the ionization potential lowering factor ΔE_{ion} should be taken into account (the typical value being on the order of 0.1 eV).

It should be noted that, by definition, LTE might also result in spatial decoupling, possibly leading to different plasma temperatures at different spatial positions. This should be considered especially when comparing results obtained in different observation geometries (Panne 1998).

1.3.2 Measurement of plasma temperature

Many methods have been described for determining the plasma temperature based on the absolute or relative line intensity (line pair ratio or Boltzmann plot), the ratio of line to continuum intensity, etc. Depending on the experimental conditions, one of these methods may be more suitable than others. For the diagnosis of early phase plasma, for instance, Liu *et al.* (Liu 1999) used the line-to-continuum intensity ratio, because line and continuum intensities are typically comparable at the start of plasma

evolution. As our main interest here is in the analytical applications of LIBS (i.e. involving observations of the plasma at later times), this method will not be discussed further.

Provided that the LTE hypothesis described above is fulfilled, the plasma temperature can be calculated from the intensity ratio of a pair of spectral lines originating in different upper levels of the same element and ionization stage. In fact, assuming that the level population obeys a Boltzmann distribution (equation (1.7)), the total spectrally integrated radiant emissivity corresponding to the transition between the upper level *i* and the lower level *j* is given by

$$e_{ij} = \left(\frac{hc}{4\pi}\right)\frac{A_{ij}g_i}{\lambda_{ij}Z^s(T)}n^s e^{-E_i/KT} \qquad 1.11$$

where λ_{ij} , A_{ij} and g_i are the wavelength, the transition probability and the statistical weight for the upper level, respectively; c is the speed of light, and the other symbols have already been defined. With the detectors typically used in LIBS measurements, an alternative formula in terms of the integrated line intensity (number of transitions per unit volume per unit time) is preferred, i.e.

$$I_{ij} = n_i^s A_{ij} = \frac{A_{ij}g_i}{Z^s(T)}n^s e^{-E_i/KT} \qquad 1.12$$

Now, by considering two lines, λ_{ij} and λ_{mn}, of the same species, characterized by different values of the upper level energy ($E_i \neq E_m$), the relative intensity ratio can be used to calculate the plasma temperature

$$T = \frac{E_i - E_m}{kln\left(\frac{I_{mn}g_iA_{ji}}{I_{ij}g_mA_{mn}}\right)} \qquad 1.13$$

When selecting a line pair, it is advisable to choose two lines as close as possible in wavelength and as far apart as possible in excitation energy. This is to limit the effect of varying spectral response of the apparatus, as well as to minimize the sensitivity to small fluctuations in emission intensity. Assuming that the intensity values are the only factors affected by the experimental error, the uncertainty in the temperature determination based on equation (1.13) can be given as

$$\frac{\Delta T}{T} = \frac{kT}{\Delta E}\frac{\Delta R}{R} \qquad 1.14$$

where $\Delta E = E_i - E_m$ is the difference in energy of the two states observed, $R = \frac{I_{ij}}{I_{mn}}$ is the measured ratio of emission intensities, and ΔR is the uncertainty associated with the ratio. As is clear from equation (1.14), large values of ΔE will minimize the effect of the uncertainty in R on the uncertainty in T (Alkemade 1982).

As we have seen, the emitted spectral line intensity is a measure of the population of the corresponding energy level of a certain species in the plasma. Under the assumptions that the plasma is both in LTE and optically thin, if we have information on the intensity emitted from several excited levels, we can then determine the temperature which is responsible for the observed population distribution. Once again, we use the Boltzmann equation (equation (1.7)) to relate the population of an excited level *i* to the total number density n^s of the species s in the plasma, and equation (1.12) to represent the intensity of the transition starting with level *i*. After linearization of expression (1.12), the familiar form of the Boltzmann plot equation is obtained

$$ln\frac{I_{ij}}{A_{ij}g_i} = ln\left(\frac{n^s}{Z^s(T)}\right) - \frac{E_i}{kT} \qquad 1.15$$

Measurement of the intensities of a series of lines from different excitation states of the same species allows evaluation of the plasma temperature, provided that the transition probabilities and statistical weights are known. A plot of the left-hand side of equation (1.15) vs. E_i has a slope of $-1/kT$. Therefore, the plasma temperature can be obtained via linear regression, without knowing n^s or $Z^s(T)$

The use of several different lines instead of just one pair leads to greater precision of the plasma temperature determination. In fact, though the precision of the intensity values can be improved by increasing the signal intensity, the transition probability values reported in the literature exhibit significant degrees of uncertainty (from 5% to 50%). The use of many lines in some sense "averages out" these uncertainties.

Because emission lines from different ionization stages are usually present in a laser induced plasma, a combination of the Saha ionization and Boltzmann excitation distributions can be used to measure the electron temperature. The most common form of the coupled Saha–Boltzmann relation takes the form of the ionic/atomic emission radiance ratio

$$\frac{e_{ij}^{II}}{e_{mn}^{I}} = \left(\frac{A_{ij}^{II} g_i^{II} \lambda_{mn}^{I}}{A_{mn}^{I} g_m^{I} \lambda_{ij}^{II}}\right)\left(\frac{2(2\pi m_e kT)^{3/2}}{n_e h^3}\right) e^{-\frac{\left(E_{ion}-\Delta E_{ion}+E_i^{II}-E_m^{I}\right)}{kT}} \qquad 1.16$$

The superscripts I and II denote atomic and ionic parameters, respectively. Here, E_{ion} is the first ionization potential and ΔE_{ion} is the lowering correction parameter. The coupledform of the Saha–Boltzmann distribution can be linearized as in the case of the Boltzmann relation and rearranged in terms of line intensity to yield

$$ln\left(\frac{I_{ij}^{II} A_{mn}^{I} g_m^{I}}{I_{mn}^{I} A_{ij}^{II} g_i^{II}}\right) = ln\left(\frac{2(2\pi m_e kT)^{3/2}}{n_e h^3}\right) - \frac{\left(E_{ion}-\Delta E_{ion}+E_i^{II}-E_m^{I}\right)}{kT} \qquad 1.17$$

Plotting the logarithmic ratio of several ionic and atomic emission line combinations as a function of their energy differences results in a line whose slope is inversely proportional to the electron temperature (when the source is in LTE). The energy difference is typically larger than the energy spread within a single ionization stage. Accordingly, the slope from a linear regression calculation is less sensitive to measurement noise. Furthermore, the electron density can now be obtained from the intercept. It should be noted that, in contrast to the Boltzmann plot alone, the intercept of the coupled Saha–Boltzmann plot does not require an absolute intensity calibration because the geometric factors cancel out in the ratio (Bye 1993).

1.3.3 Two methods of quantitative analysis.

Quantitative LIBS analysis is conventionally performed using calibration-based method in which calibration curves of emission line intensity vs. elemental concentration using a few matrix-matched reference samples with known composition are first produced and then the composition of an unknown sample is found by comparing the analyte line signals with spectral intensities obtained from the

calibration curves (Cremers 1987; Russo 1995; Chan 1991; Mao 1996; Russo 2002; Mao 1998). The applicability of this method is limited because a matrix with composition similar to the unknown sample is required which is, in many cases, not possible. As an alternative method, the calibration-free LIBS (CF-LIBS) method has been initially developed by Ciucci *et al* (Ciucci 1977) for quantitative elemental analysis with LIBS spectra. In this method, the elemental composition of a sample is determined from the LIBS spectrum using computational methods in analysing the basic physics of the plasma process by estimating the plasma temperature and electron number density, assuming that the plasma composition represents exactly the composition of the sample, i.e. stoichiometric ablation, and the plasma is optically thin and is in local thermodynamic equilibrium (LTE). For the quantification of elemental contents in the sample, algorithm is developed relating the experimentally measured spectral intensity values at a time delay where the plasma is optically thin and in LTE, with the basic physics parameters of the plasma. Since its inception, the CF-LIBS method has been used by several research groups across the world to analyse metallic alloys, such as Al-based (Nouvellon 1999; Borisov 2000), Fe-based (Borisov 2000), Cu-based (Dudragne 1998; Cremers 1983; Tran 2001; Cremers 1985; Carranza 2002) and Au-based (Tran 2001, Semerok 1999) alloys as well as non-metallic samples, such as soil, rock and glass (Tran 2001, Semerok 1999; Cheung 1997; Thorne 1974; Panne 1998). The advantage of this method is that the need for a matching matrix, a serious problem in the calibration-based LIBS, is overcome. A major drawback of this method is that one needs to detect at least one line of each element in the plasma with known atomic data. Gomba *et al* (Liu 1999) have developed a CF-LIBS procedure different from that of Ciucci *et al* (Nouvellon 1999) to quantify the contents of the elements by estimating the plasma temperature, electron number density and relative number densities of the neutral and singly ionized ionic species in the LIBS plasma using the experimental spectral line intensity values in the time window where the plasma is optically thin and in LTE.

For quantitative elemental analysis from the LIBS spectral line intensities, it is essential to characterize the time-evolving LIBS plasma in terms of its temperature and electron number density and find out the

time window where the LIBS plasma is optically thin and in LTE. Based on plasma spectroscopy, the Boltzmann plot method yields the temperature T and the Saha–Boltzmann equation method yields the electron number density n_e of optically thin plasmas in LTE.

1.4 Principal Component Analysis (PCA): the statistical method used in this study

It is more than a century since Karl Pearson invented the concept of Principal Component Analysis (PCA). Nowadays, it is a very useful tool in data analysis in many fields. PCA is the technique of dimensionality reduction, which transforms data in the high-dimensional space to space of lower dimensions. PCA algorithms were used in this study for reducing the high-dimensional spectroscopic data by constructing a linear combination of the original variable into a few orthogonal principle components which contain most of the variability of the data set. This projection method allows first visualization of the natural clustering in the data, second primary evaluation of the between-class similarity and thirdly finding the reasons behind the observed pattern by making correlation with the chemical or physico-chemical properties of the studied samples.

Indeed, PCA itself does not reduce the dimension of the data set. It only rotates the axes of data space along lines of maximum variance. The axis of the greatest variance is called the first principal component. Another axis, which is orthogonal to the previous one and positioned to represent the next greatest variance, is called the second principal component, and so on. The dimension reduction is done by using only the first few principal components as a basis set for the new space. Therefore, this subspace tends to be small and may be dropped with minimal loss of information.

Originally, PCA is the orthogonal transformation which can deal with linear data. However, the real-world data is usually nonlinear and some of it, especially multimedia data, is multilinear. Recently, PCA is not limited to only linear transformation. There are many extension methods to make possible nonlinear and multilinear transformations via manifolds based, kernel-based and tensor-based techniques. This generalization makes PCA more useful for a wider range of applications. Determining

this fact allows an experimenter to discern which dynamics are important, which are just redundant and which are just noise.

Principal Component Analysis (PCA) has been called one of the most valuable results from applied linear algebra. It is a useful statistical technique that has found application in fields such as face recognition, image compression, neuroscience, computer graphics and is also a common technique for finding patterns in data of high dimension. It is a way of expressing the data in such a way as to highlight their similarities and differences. Since patterns in data can be hard to find in data of high dimension, where the luxury of graphical representation is not available, PCA is a powerful tool for analyzing data (I.T. Jolliffe, 2002).

2

Coffee

2.1 Introduction

In this chapter we will discuss about the species of coffee, the relationship between coffee quality and coffee processing, the composition of coffee, different methods of elemental analysis in coffee and about the discrimination of coffee.

Coffee has been for decades the most commercialized food product and most widely consumed beverage in the world. Since the opening of the first coffee house in Mecca at the end of the fifteenth century, coffee consumption has greatly increased all around the world. In 2010, coffee production reached 8.1 million tons worldwide. It is the second most important exported commodity in the world after oil (Pendergrast 1999).

The reasons for this continuous increase in coffee consumption include improved cup quality through selection of varieties and breeding, better agricultural practices; the creation of specialty shops, and a change in coffee's image through the dissemination of information on the health benefits of long-term coffee consumption. Today, coffee is considered a functional food, primarily due to its high content of compounds that exert antioxidant and other beneficial biological properties. The characteristic flavor and richness of coffee aroma make it a unique beverage, with almost a thousand volatile compounds identified in roasted coffee (Yeretzian 2003).

More than 80 developing countries mainly earn their foreign currency from coffee. Ethiopia is one of the eight regions in the world considered to have a strikingly high level of diversity in cultivated crop plants (Vavilov 1951). Arabica coffee (*Coffea arabica* L.) is one of the crops which have their origin and centre of diversification in Ethiopia. The domestication and use of coffee in Ethiopia dates back some 2000 years ago (Luxner 2001). For Ethiopia, coffee is 4-5% of the GDP, 20% of the government

revenue, 60% of the total foreign exchange earnings up to the year 2000 and a livelihood for more than 25% of its population (Tafesse 1996).

2.2 Map of coffee growing regions in Ethiopia

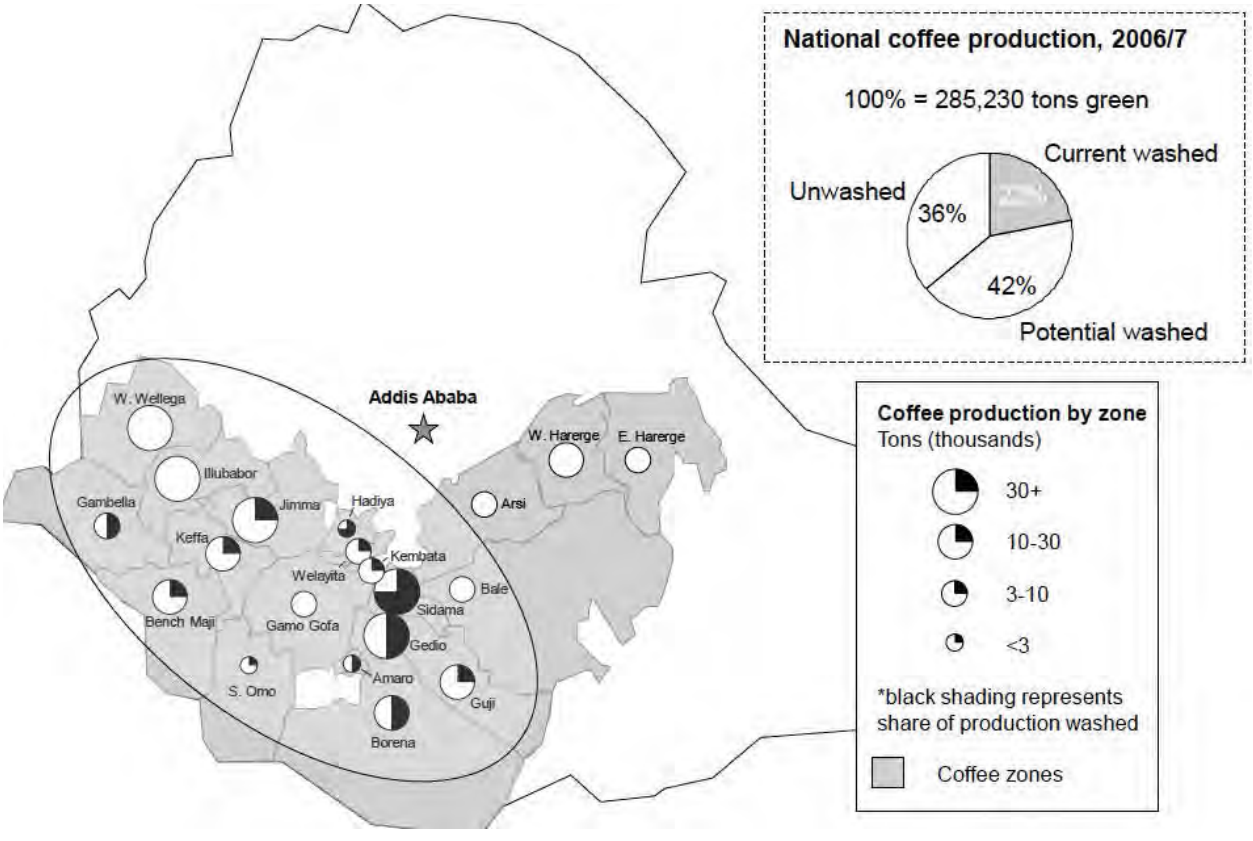


Figure 2.1: coffee growing regions in Ethiopia

2.3 Coffee Species

The coffee tree belongs to the Rubiaceae family, genus *Coffea*. Although more than 80 coffee species have been identified worldwide (Clarke 2003), only two are economically important. *Coffea arabica,* also known as Arabica coffee, is responsible for approximately 70% of the global coffee market, and *Coffea canephora* or Robusta coffee accounts for the rest (ICO 2009 2011; ABIC, 2011). Arabica and Robusta coffees are different in many ways, including their ideal growing climates, physical aspects, chemical composition, and characteristics of the brew made with the ground roasted seeds.

2.4 Coffee processing

There are two major coffee processing styles in the world: Sun-dried natural, and fully washed. Certain coffee processing styles are more prevalent in certain regions, but in general it is possible to find both styles throughout the coffee growing regions in Ethiopia. Many countries have one national processing style, either washed (example: Colombia) or natural (example: Haiti). Ethiopia has both, and both on a large scale.

Processing refers primarily to the method of removing the skin, pulp, and parchment from coffee cherry, to reveal the green coffee bean (actually the seed of the plant) underneath. The manner in which this is done has a huge impact on the flavor of the resulting coffee. Coffee pulp, or mucilage, is very sticky and dense in sugars. Special processes are needed to remove the mucilage from the beans. These general categories (washed vs. natural) are common throughout the coffee-producing world. However, the specifics of each process can vary considerably from country to country.

In Ethiopia, Sun-dried natural processing is usually done using raised drying beds, though some coffees are also dried on the ground, especially coffees for the local market. Raised beds made out of wood posts, about waist-high, are covered in a material like burlap or nylon netting. Producers lay the coffee cherries, skin and all, out to dry on the beds.

Over time, the skin and sticky juices of the cherries dry out in the sun. This process can take several days to a few weeks, depending on the temperature and the intensity of the sun. During the drying process, the cherries shrink in size and eventually become hard and completely dry. Once the process is completed, sacks of dried cherries are taken to a hulling station for the removal of the outer cherry.

Care must be taken to ensure even drying of cherries, and to avoid any contact between the cherries and contaminating substances, like direct contact with soil. Insufficient attention to these details can lead to muddy, dirty, or fermented flavors in the cup.

The great advantage of natural processing is that it does not require any water, nor any elaborate machinery or facilities. As a result, one finds more naturally processed coffees in drier areas, as well as poorer or more remote areas.

Generally, as the result of prolonged and sun-fueled contact with the cherry's own natural sugars, sun-dried natural coffees have a sweet, fruity character with a creamy mouth feel. The best, most-carefully cared-for sun-dried natural coffees can have intense berry flavors, tropical fruit aromatics, and chocolaty undertones. Natural-process green coffee beans often have a yellowish or orange-like tinge to them. This comes from prolonged contact with the sugars as they "cook" into the bean in the sunlight.

In the washed or "fully washed" style of processing, the outer skin of the coffee cherry is removed immediately after harvesting, usually the same day the cherries were picked. This is done using machines which "pick" or scrape away just the very outer layer of the cherry, leaving behind the parchment coffee covered in sticky mucilage.

The "washed" designation refers to what happens to the coffee next. The mucilage-coated beans are then fermented with water in large tanks, usually cement. The process of fermentation breaks down the sugars in the mucilage and frees it from the parchment. Fermentation usually takes around 24 hours, though shorter or longer fermentation times are possible, depending on the local climate, altitude, and other factors.

Once fermentation is complete, the coffee is released from the fermentation tank and pushed manually, with the help of some flowing water, down long channels. This agitation frees up any remaining mucilage and separates it from the parchment coffee. At the end of the channels, the coffee enters another tank where it is rinsed with fresh water. The result is wet coffee in parchment, free of the sticky mucilage.

From the final washing tank, the wet parchment coffee is taken to dry in the sun, usually on raised beds. This process of drying happens quickly, because there is no skin or mucilage between the sun and the parchment. After one or two days in the sun, the coffee is removed from the beds and stored in sacks in a

warehouse. When it is to be exported, the coffee is usually taken to a larger central mill where the parchment is removed, and the coffee is sorted and bagged for export.

The disadvantage of the washed process is that it requires large quantities of water and more infrastructure. In many locales, it is simply not feasible to do the washed process. Washed coffee tends to have a clarity of flavor and aroma that is often lacking in natural coffees. Many cuppers assert it is easier to taste the influence of soil and varietal in washed coffees. Acidity comes through more clearly, and the cup is generally cleaner. The cleanest, highest quality, high-altitude washed coffees can have an intensely refreshing character (Willem J. Boot 2011).

2.5 Composition of Coffee

The coffee beverage can be consumed for many reasons, including its stimulatory effects resulted from the presence of caffeine, health benefits, and primarily excellent taste and aroma (Grembecka et al. 2007; Butt and Sultan 2011; Oliveira et al. 2012). Due to a habitual consumption of coffee, its chemical composition, namely the presence of essential, non-essential and toxic elements, has to be known and kept under control in terms of its safety, and to assist its quality, nutritional value, and certain sensorial properties (Krivan et al. 1993; De Nadai Fernandes et al. 2002; Zaidi et al. 2006; Filho et al. 2007; Oleszczuk et al. 2007; Oliveira et al. 2012). Coffee is composed primarily of carbohydrates and fiber, proteins, lipids, minerals, organic acids, chlorogenic acids, trigonelline, and caffeine.

2.6 Elemental Analysis of Coffee

Although the content of elements in coffee is only about 5 % , it seems to be a good indicator of the coffee authenticity. Apparently, it can bring the useful information about individual elemental patterns that are distinctive to the origin of growing soils for coffee plants in addition to cultivation and environmental conditions used. The elemental analysis of coffee by means of instrumental measurement methods may have other uses. It can be used to prove the high quality and safety of coffee beans. Commonly, different atomic absorption and emission spectrometry and instrumental neutron activation analysis methods are used to determine concentrations of various elements in green, roasted, and ground

or instant coffees and coffee infusions, but these samples have to be suitably prepared prior to the analysis.

The authenticity of coffee related to its certain geographical origin and botanical variety based on the elemental analysis and statistical pattern recognition methods seems to be quite important to producers and consumers (Krivan et al. 1993; dos Santos and de Oliveira 2001; De Nadai Fernandes et al. 2002; Fernandes et al. 2005; Filho et al. 2007; Grembecka et al. 2007; Oleszczuk et al. 2007; Bertrand et al. 2008). Since consumers usually search for coffee with special taste and aroma, of a high quality and produced from beans of known variety and origin, the determination of the provenience of coffee is a very important part of the coffee trade (Martin et al. 1998a; Vega-Carrillo et al. 2002; Bertrand et al. 2008). The quality of Ethiopian coffee is determined by two main factors namely the geographic origin and the post harvest processing (Nicholas, 2007). Sometimes dishonest producers can sell cheaper varieties or blends of coffees but recommending them as of a much better quality (Anderson and Smith 2002). The difference between the declared and the real composition can also be the effect of an accidental mislabeling (Martin et al. 1999). In all these cases, the reliable and dependable analysis of selected components or properties of coffee may exclude such mistakes, i.e., fraudulent or accidental mislabeling and confirm the high quality of the final product (Suseela et al. 2001).

To guarantee stated quality and safety of a final coffee product and protect well-being and health of consumers, different parameters responsible for the wholesomeness of green beans, roasted beans, prepared coffee and its infusions have to be measured using suitable analytical methods. For example, in the organoleptic analysis of green coffee beans, their odor and taste in addition to the information about their size, shape, color, and cross-section are ascertained as a part of the quality assessment (Belitz et al. 2009). Color and flavor characteristics are important to find the best degree of roasting green beans (Belitz et al. 2009). For the evaluation of the quality of coffee infusions, the flavor of prepared beverages is commonly described under standardized conditions (Sanz et al. 2002). All individual notes

of each sample are collected and its unique profile is assessed, however, it should be noted that opinions of qualified coffee testers on coffee taste and aroma can be subjective (Krivan et al. 1993; Anderson and Smith 2002).

Chemical methods of the coffee analysis are similar to those used in the food quality control and assessment (Martin et al. 1998a). They are based on the determination of different compounds, e.g., volatile compounds, caffeine, tannins and polyphenols, lipids, individual carbohydrates like sucrose, glucose, fructose, arabinose, galactose, polysaccharides like cellulose, amino acids, vitamins B3 and PP, chlorogenic acid, trigonelline, and minerals (Bernal et al. 1996; Costa Freitas and Mosca 1999; Anderson and Smith 2002; Villarreal et al. 2009; Hecimovic et al. 2011;Wei et al. 2011). These chemical species are often measured for the purpose of discriminating coffee varieties and brands or determining the coffee origin (Bernal et al. 1996; Costa Freitas and Mosca 1999; Anderson and Smith 2002; Villarreal et al. 2009; Hecimovic et al. 2011). However, it should be considered that all stages involved in the production of coffee, from coffee harvesting to roasting, can change the composition of the final product (Anderson and Smith 2002; Mussatto et al. 2011). Among different substances present in coffee, only caffeine is stable to the excessive roasting temperature (Mussatto et al. 2011). Other chemical compounds are susceptible to degradation during production and storage conditions (Anderson and Smith 2002). Hence, a reliable and independent method enabling to differentiate the geographic growing origin of coffee has to be focused on compounds that are stable during all coffee production stages and a subsequent storage. Elements and their concentrations fulfill this requirement and for that reason the elemental analysis of coffee, aimed at determining its elemental composition is so important for the purpose of its quality control and bromatological value evaluation (Krivan et al. 1993; Anderson and Smith 2002).

2.7 Instrumental Methods of Analysis

When considering enormous coffee production and consumption in the world, the determination of elements in coffee, including those regarded as nutrients and those classified as toxic and hazardous to

health, is certainly of great interest and importance (Dos Santos and de Oliveira 2001; Oleszczuk et al. 2007). Hence, the development of instrumental methods suitable for a reliable elemental analysis of coffee, providing measurements of concentrations of mineral nutrients and concomitant traces, is essential for the whole coffee sector because competently assures the high quality of the final product (Ribeiro et al. 2003; Tagliaferro et al. 2006).

Flame atomic absorption spectrometry (FAAS) with a deuterium lamp background corrector or intermittently with a Zeeman effect background corrector (Filho et al. 2007) is quite often used for selective determinations of different major (Ca, K, Mg, Na), minor (Cu, Fe, Mn, Zn) and trace (Cd, Co, Cr, Ni, Pb) elements of coffee (Krivan et al. 1993; Onianwa et al. 1999; Anthemidis and Pliatsika 2005; Filho et al. 2007; Grembecka et al. 2007; dos Santos et al. 2009, 2010; Ashu and Chandravanshi 2011). High-resolution continuum source flame atomic absorption spectrometry (HRCS- FAAS) can also be used for this purpose (Ca, Fe, K, Mg, Mn, and Na) (Oliveira et al. 2012). Concentrations of K and Na are often measured by flame atomic emission spectrometry (FAES) using separate photometers (Filho et al. 2007) or the same instruments as for FAAS but working in the emission mode (Ashu and Chandravanshi 2011).

Unfortunately, FAAS is recognized to be not sensitive enough to quantify some important trace elements (Grembecka et al. 2007). The latter elements are preferred to be determined using inductively coupled plasma optical emission spectrometry (ICP-OES), i.e., Cd, Cr, Ni, and Pb, (dos Santos et al. 2010) or differential pulse anodic stripping voltammetry (DP-ASV), i.e., Pb, Cd, and Cu (Suseela et al. 2001). Samples of green, roasted, or instant coffees require being appropriately prepared before measurements by the digestion and the mineralization of their organic matrix. The calibration of FAAS is commonly carried out using simple standard solutions (Onianwa et al. 1999; Suseela et al. 2001; Anthemidis and Pliatsika 2005; Filho et al. 2007; Grembecka et al. 2007; dos Santos et al. 2009, 2010; Ashu and Chandravanshi 2011; Oliveira et al. 2012). Exceptionally, in case of measurements of K and Na, a solution of CsCl3 can be added to standards and samples as an ionization buffer (chemical suppressor)

(Grembecka et al. 2007; Oliveira et al. 2012). In a similar way, La salts, i.e., solid La(NO3)3 (Ashu and Chandravanshi 2011) or a solution of LaCl3 (Grembecka et al. 2007), are added to standard and sample solutions to prevent chemical interferences in the quantification of Ca and Mg

Electrothermal atomic absorption spectrometry (ETAAS) with the deuterium background (Oleszczuk et al. 2007) or Zeeman correction (Krivan et al. 1993; Magalhaes et al. 1999; Anthemidis and Pliatsika 2005) is less frequently used. High-resolution continuum source graphite furnace atomic absorption spectrometry (HR-CS-GFAAS) can alternatively be applied (Oliveira et al. 2012). Both aforementioned techniques are primarily used to determine trace and minor elements of coffee, i.e., Al, Co, Cr, Cu, Fe, Ni, Mn, and Sr. Similarly as for FAAS, samples of coffee to be analyzed have to be digested and this results in releasing elements into solutions in the form of simple ions (Magalhaes et al. 1999; Oliveira et al. 2012). Interesting approaches to measurements of elements by means of ETAAS without the initial digestion of samples have also been reported and rely on the direct analysis of solid samples (Oleszczuk et al. 2007) or their slurries (Magalhaes et al. 1999). As compared to the analysis of solutions of digested samples, the latter methods certainly offer a very high sensitivity for trace elements due to the absence of any sample dilution as well a minimum risk of the contamination (lower blanks) or losses of elements due to reduced amounts of reagents used for the preparation of samples.

ICP-OES is very often applied in the elemental analysis of coffee samples. It is especially attractive and helpful in determinations of a number of elements, including major (Ca, K, Mg, Na, P, S), minor (Al, B, Co, Cu, Fe, Mn, Sn, Zn) and trace elements (As, Ba, Cd, Cr, Ni, Pb, Sb, Se, Si, Sr) (dos Santos and de Oliveira 1997, 2001; Martin et al. 1996, 1998a ,1999; Jaganyi et al. 1999; Jaganyi and Madlala 2000; Anderson and Smith 2002; Ribeiro et al. 2003; Anthemidis and Pliatsika 2005; Fernandes et al. 2005; Oleszczuk et al. 2007; Bertrand et al. 2008; Santos et al. 2008; Castro et al. 2009; Frankova et al. 2009; Tezotto et al. 2012). Lower detection limits, higher sensitivities, wider linear dynamic ranges, and faster measurements are additional advantages of ICP-OES over other atomic spectrometry methods, i.e., FAAS and ETAAS, commonly used in multi-element analyses of coffee (dos Santos and de Oliveira

1997). Samples are digested prior to the determination of elements by ICP-OES as well, however, a flow injection (FI) s ystem f or a n o n-line s ample s lurry f ormation of s olid s amples (5–50 m g) w ithout a decomposition step has been proposed to improve the analysis and shorten its duration (Anthemidis and Pliatsika 2005).

Other spectrometric methods, including inductively coupled plasma mass spectrometry (ICP-MS), direct current p lasma o ptical em ission s pectrometry (DCP-OES) o r to tal reflection X -ray f luorescence spectrometry (TXRFS), ar e much rarely u sed f or the an alysis of coffee. I CP-MS h as b een u sed t o determine some selected elements (Cd, Cr, Cu, Mn, Ni, Pb, U, Zn) in coffee samples after their brewing and the later mineralization of infusions prepared (Santos et al. 2004). DCP-OES has been applied for the determination of Al in samples of coffee and their infusions (Rajwanshi et al. 1997).

In general, the chemical composition of roasted and ground coffee is mostly and closely related to the growing origin of coffee beans, a factor primarily associated with soil conditions, the coffee variety, and the cultivation method of coffee plants (Jaganyi and Madlala 2000; Anderson and Smith 2002; Filho et al. 2007; dos Santos et al. 2010; Ashu and Chandravanshi 2011). Procedures included in the processing of green and roasted coffee beans or even brewing methods used for ground coffee are also important (Jaganyi and Madlala 2000; Grembecka et al. 2007). Differences in levels of macro- and micronutrients found i n g reen an d r oasted ar abica co ffees a re m arked and r oasted co ffees h ave u sually higher concentrations of K, Na, Ca, Mg and Fe as compared to unprocessed coffee of that variety (Filho et al. 2007). Although the chemical composition of arabica and robusta coffees is similar, it seems that one of the b est cr iterions t o d ifferentiate t hem i s t he el emental co mposition, b ecause el ements i n t he co ffee commodity are s table w hile differences i n t heir c oncentrations a re more di stinctive t han t hose established for various organic substances (Krivan et al. 1993; Martin et al. 1998a, 1999; Grembecka et al. 2007). the concentration of major and minor elements is quite varied and this is associated with a vast influence of the origin (especially the type of soil where coffee plants are cultivated), the variety and the type of coffee, pr ocesses i nvolved i n t he pr oduction of n atural o r s oluble c offees a nd m eans of t he

confection and the storage of coffee (dos Santos and de Oliveira 2001; Vega-Carrillo et al. 2002; Zaidi et al. 2006; Grembecka et al. 2007; dos Santos et al. 2010; Ashu and Chandravanshi 2011).

2.8 Discrimination of Coffee

Elements are only about 5 % of the total weight of coffee but they seem to be very good indicators of its origin and variety, the type of soil on which coffee plants are cultivated and environmental and agricultural growing conditions, especially the farming method used (Anderson and Smith 2002; Szefer 2007; Bertrand et al. 2008; Gonzalvez et al. 2009; dos Santos et al. 2010). Principally, the content of various elements in coffee plants has a strong correlation with the growing environment, which is strictly associated with the soil characteristics (the content of lime, the presence of the organic material, the soil pH, the drainage status of the soil) and the influence of weather conditions (Krivan et al. 1993; Anderson and Smith 2002; Bertrand et al. 2008). Hence, the elemental composition of coffee beans and certain coffee products coming from specific places and regions are very distinctive (Krivan et al. 1993; Gonzalvez et al. 2009; dos Santos et al. 2010).

Since it is recognized that Arabica are coffees of the highest quality, undisputable methods enabling to distinguish coffees of different origin and variety within a country area are very important (Martin et al. 1998a, b, 1999; Bertrand et al. 2008). The statistical analysis of the data representing the elemental composition is a very powerful approach to such quality control and assessment of the coffee genuineness (Martin et al. 1998a, b, 1999; Bertrand et al. 2008). Treating analyzed coffee samples as objects and concentrations of elements determined in these samples as variables, it is possible to establish individual elemental patterns within these objects and classify them according to the geographical origin, the variety or the type of coffee by means of different chemometric techniques (Martin et al. 1998a, b, 1999; Fernandes et al. 2005; Szefer 2007).

Major and minor elements that are convenient for the efficient discrimination between green and roasted coffees are Ca, Cu, Fe, K, Mg, and Na (Filho et al. 2007). Different types of solid coffees, i.e., ground

and instant, Arabica and Robusta, and their infusions can successfully be differentiated using concentrations of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, and Zn (Grembecka et al. 2007).

Unfortunately, the correlation between the content of elements in coffee samples and their geographical origin is sometimes problematic (Fernandes et al. 2005). This is because a lot of coffees marketed are blends of coffees from different regions. It is recognized, that the effect of the location and the origin of coffee is usually significant for the content of B, Ca, Cu, Mg, Mn, P, and Zn, revealing the potential of the elemental analysis for the discrimination of territories within a given country and the reflection of both climatic and soil diversities (Bertrand et al. 2008).

In addition, when processing the data with different statistical tools for the purpose of the classification of coffee samples, it should be considered that several factors may affect the elemental composition of coffee beans and the quality of roasted and/or ground coffees (Vega-Carrillo et al. 2002; Tagliaferro et al. 2007; dos Santos et al. 2010; Tezotto et al. 2012). Since the coffee cultivation requires fertilizers as a valuable source of macro- (K, N, P, and S) and micronutrients (B, Cu, Fe, Mn and Zn) for the proper growth of coffee plants, inorganic fertilizers (mainly phosphates) and organic residues (pulps or husks from the coffee processing) may result in the contamination of the crop soil with trace elements and their accumulation (Tagliaferro et al. 2007; dos Santos et al. 2010). Taken up by coffee plants, these elements can reach coffee beans and contaminate the final product (Tagliaferro et al. 2007; dos Santos et al. 2010).

Commercial fertilizers used in traditional and technological plantations of coffee usually possess very high concentrations of some toxic elements (Al, Cd, Cr, Ni, and Pb) and micronutrients (Cu, Mn, Na, and Zn) that can be taken up by coffee plants (dos Santos et al. 2009, 2010). Organic manures used in organic coffee farms, although do not contain Cd and Pb, commonly contain relatively high amounts of Cu, Cr, and Zn and significant amounts of Mn and Ni (dos Santos et al. 2009, 2010). The application of these fertilizers usually results in an increase in the content of aforementioned elements in the crop soil. In a consequence, cultivated coffee plants and beans may contain higher levels of Al, Cd, Cu, Na, and

Zu (Fernandes et al. 2005; dos Santos et al. 2009, 2010). Differences in the uptake of elements from naturally fertilized soils and those fertilized with different inorganic agricultural chemicals are significant and commonly reflected by the elemental composition of coffee beans (dos Santos et al. 2010). Accordingly, strong correlations between concentrations of Ca, Cd, Cr, Cu, Mg, Fe, Mn, Ni, Pb, and Zn in organic residues used as fertilizers and amounts of these elements in organic coffee beans are reported (dos Santos et al. 2009, 2010).

In our study, as it can be referred from section 4.2 we have compared the results of our study with couple of previous works and found it in good agreements.

3

Experimental methods for coffee

3.1 Descriptions of Coffee Varieties Studied

In this section we will briefly give the descriptions of the coffee samples studied in our researches.

Seven Ethiopian export quality coffee and their ten mixtures are analyzed by using LIBS technique. The names of the samples are shown in the table 3.1 below

No	Nomenclature	Types of Coffee
Sample 1	(1S1, 1S2, 1S3)	UNWASHED HARAR
Sample 2	(2S1, 2S2, 2S3)	UNWASHED LEKEMTE
Sample 3	(3S1, 3S2, 3S3)	WASHED LIMU
Sample 4	(4S1, 4S2, 4S3)	HARAR BLEND
Sample 5	(5S1, 5S2, 5S3)	WASHED SIDAMA
Sample 6	(6S1, 6S2, 6S3)	UNWASHED SIDAMA
Sample 7	(7S1, 7S2, 7S3)	WASHED YIRGACHEFE
Example of mixtures	(75S1, 75S2, 75S3)	Mixture of sample 5 and sample 7

Table 3.1: The different samples studied with the chosen nomenclature

Harar Coffee

The region of Harar is found in eastern part of Ethiopia. Practically all coffee from Harrar is sun-dried natural. There are several heirloom varietals that grow specifically in this region, that interact well with the altitude, climate, and soil type to produce a very unique flavor profiles. Quality Harrar coffees are

notable for a fruity characteristic and a creamy body. The finest Harrar coffees have a distinct note of blueberry, though many other fruity and fruit-like aromatic flavors can occur.

Harrar coffee is exported all over the world, but there is a particular demand for it in Saudi Arabia. This constant demand tends to keep the price for commercial grade Harrar coffee slightly higher than most other Ethiopian coffee regions.

Harrar coffee — all of which is unwashed — is available in specialty grade and commercial grade. Commercial grade coffees are given a grade between 3 and 9, and are designated geographically by the letters A, B, C, and D. Remember, the letters do not represent grades, only geographical categories.

Sidama Coffee

The region of Sidama is in southern Ethiopia. Sidama features an extraordinarily wide variety of coffee flavors. Many different grades of both washed and unwashed coffees are produced, and there can be striking differences from town to town. Varying soil types, micro climates, and especially the countless heirloom coffee tree varietals make for a kaleidoscope of different flavors. It is difficult to make any single description of Sidama coffees, without immediately encountering another coffee that fits a completely different profile. The strength of Sidama coffee lies in its variety.

One feature of excellent Sidama coffee is often a profound complexity. This derives from the many different heirloom varietals. Many different farmers and pickers, each with a very small patch of land, often with their own unique varietals, will pool their coffees at a cooperative. The resulting "blend" is a unique expression of the complexity of the horticulture in the surrounding area.

High grade unwashed Sidama coffees are known for their intense fruity characteristics, while being of somewhat lighter body than unwashed Harrar coffees, for example. Another striking characteristic of Sidama coffees is that even the washed coffees often retain a salient fruity characteristic, while having much more clarity and brightness than their unwashed counterparts. Excellent coffees of many different profiles can be found in all corners of Sidama. Sidama coffees are given three tags: a grade, a geographical letter designation, and designation as washed or unwashed

Yirgacheffe Coffee

Yirgacheffe is a small micro-region within the much larger region of Sidama. However, Yirgacheffe coffees are so distinct and so well-recognized internationally that they are grouped into their own special category. Top grade Yirgacheffe coffees share many characteristics with the best Sidama coffees. Fruit flavors, a bright acidity, and a silky mouth feel are some of its hallmarks. Yirgacheffe produces both washed and unwashed coffees. While it originally became famous mostly for its washed coffees, recent years have seen the export of some highly sought after top-rate unwashed coffees as well.

Top grade washed coffees from Yirgacheffe are renowned for bright citrus acidity, often with a lemony character, with excellent sweetness. The other hallmarks of the coffee are a light, herbaceous quality that compliments the fruit flavors well, for a complex and flavorful coffee. The best unwashed coffees from Yirgacheffe often retain a high degree of acidity, with softer fruit flavors and sometimes berry characteristics.

Limu coffee

It grows in the southwest of Ethiopia between 3,600 and 6,200 feet. Limu coffee (all washed) generally has a milder acidity than Sidama and Yirgacheffe; the flavor is generally characterized by a balanced and clean cup. Traditionally, Limu coffees marketed under the name washed; the unwashed Limu coffees have normally been offered under the Jimma category.

Lekemte Coffee

Lekempti is a region located within the state of Wellega in western Ethiopia. Coffee processing styles in Wellega have traditionally been sun-dried natural. The coffee is known for its large bean size, and the flavor can have a pronounced perfume-like aftertaste (Willem J. Boot; **2011**).

3.2 Samples preparation of coffee

The seven roasted coffee samples bought from Ethiopian coffee authority grounded by using grinding machine with grain size ~20 μm. About 300 mg (fig 3.1) of each sample were pelletized using a hydraulic press with a pressure around 2 tons/cm^2 to produce an intermediate thick pellet samples. Ten mixtures of samples were also prepared by mixing 150mg of each sample. For each sample three pellets were prepared to check the repeatability of the experiments. The preparation of the powders into pellets was necessary to have better ablation efficiency and higher repeatability of LIBS measurements.

Figuer 3.1 Pellates of coffee above after analysis and lower before analysis

3.3 Experimental set up

The experimental setup consists mainly of a laser source and a system for the detection of the radiation emitted by the plasma, interfaced with a PC. In the experimental configuration (shown in figure 3.3) we use a standard Nd:YAG laser (Brilliant, Quantel) to generate the plasma. It was running in its second harmonic at 532 nm, at a repetition rate of 2 Hz. Individual laser pulses had a pulse length of 4 ns. The pulse energy used was 30 mJ. A mirror is used to direct the laser beam onto the target surface. It is inclined at an angle of 45° to the direction of incident laser

beam. The laser light was focused by a lens of 10 cm focal length. The distance between the plasma plume and the entrance slit of the monochromator (a JobinYvon THR 1500 monochromator equipped with a 1200 grooves mm^{-1} grating and resolution of 0.0015 nm) was 40 cm. The sample pellet was moved during the spectral accumulation in such way that each laser shot had a fresh sample surface. Light from the plasma was collected by a lens of 10 cm focal length placed at the same distance from the plasma plume and the entrance slit of the monochromator, under this condition the image magnification was 1:1. An ICCD (Intensified Charge Coupled Device) detector (Andor Technology, model DH520- 25F-03) was employed for spectral acquisition. Synchronization between the laser and the ICCD detector was ensured by microcomputer (Lab PC) via a pulse delay generator (Model DG 535, Stanford Research Systems, Inc). The detection system offered a spectral range between 200 and 800 nm. The microcomputer was equipped with software for data acquisition and plotting spectra. The selected spectrum was processed by averaging the signal over twenty successive laser shots. Due to this, we had a good signal-to-noise ratio. Then it was verified that the plasma was reproducible by recording the same average spectrum several times. The spectra were recorded with a delay of 1 μs after the laser pulse and a gate width of 10 μs. The delay time is important because as we have mentioned previously, at the earliest time, the plasma light is dominated by a "white light" continuum that has little intensity variation as a function of wavelength. This light is caused by bremsstrahlung and recombination radiation from the plasma as free electrons and ions recombine in the cooling plasma. If the plasma light is integrated over the entire emission time of the plasma, this continuum light can seriously interfere with the detection of weaker emissions from minor and trace elements in the plasma. For this reason, LIBS measurements are usually carried out using time-resolved detection. In this way the strong white light at early times can be

removed from the measurements by turning the detector on after this white light has significantly subsided in intensity but atomic emissions are still present. The gate width is the time period over which the light is recorded as shown in Figure 1.4a.

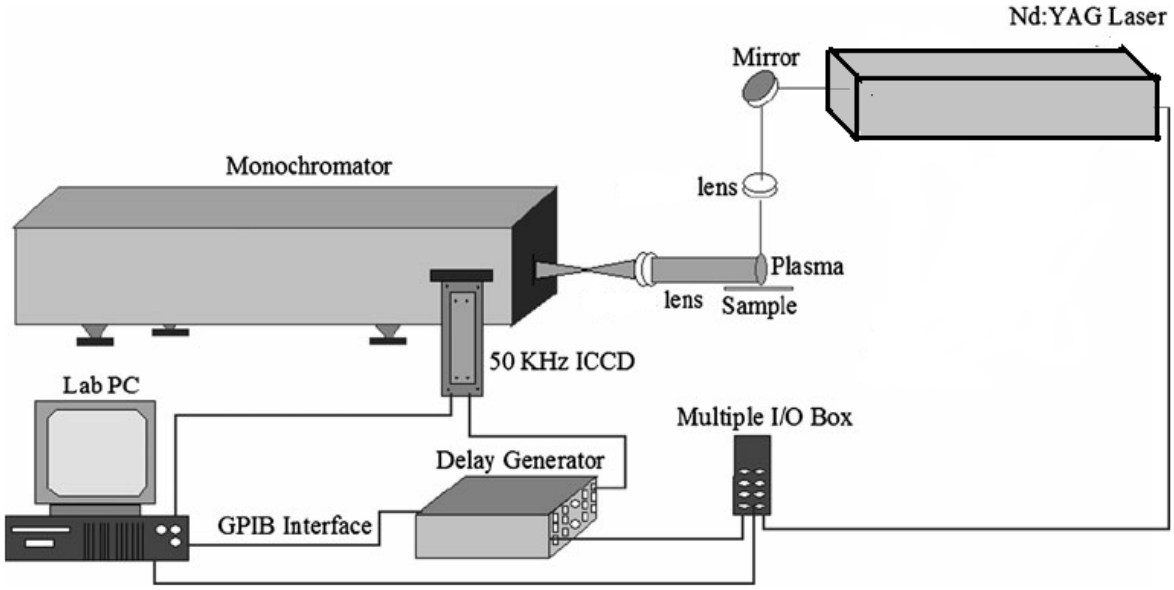


Figure 3.3: Schematic LIBS set up used at the LSAMA laboratory

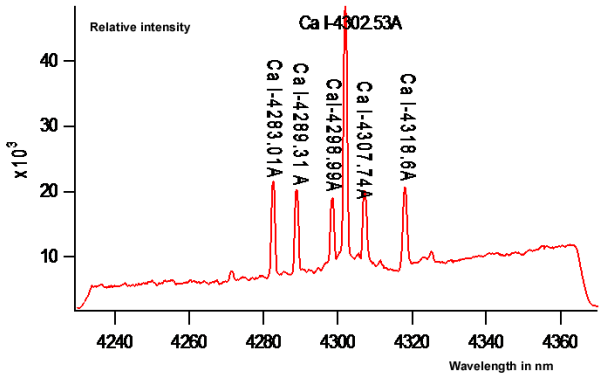
Figure 3.4: LIBS set up used at the LSAMA laboratory

4

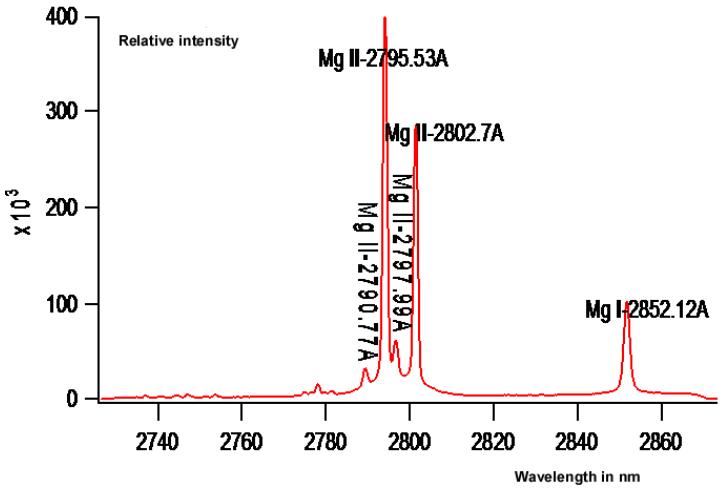
Results and discussions of coffee

4.1 Elemental variations of coffee samples in terms of integrated intensity of emission lines

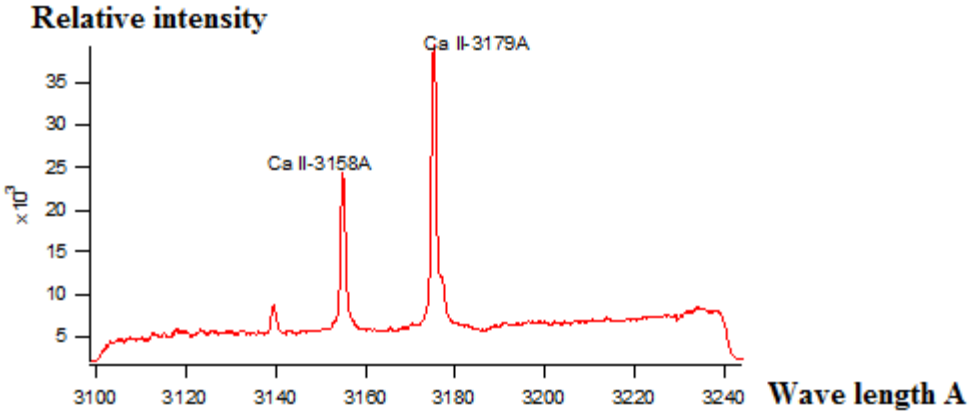
The LIBS spectrum of coffee samples were collected in wavelength range 200-800 nm in air atmosphere. Spectra acquired by LIBS are processed using Igor software. We have displayed part of the spectra in figure 4.1.



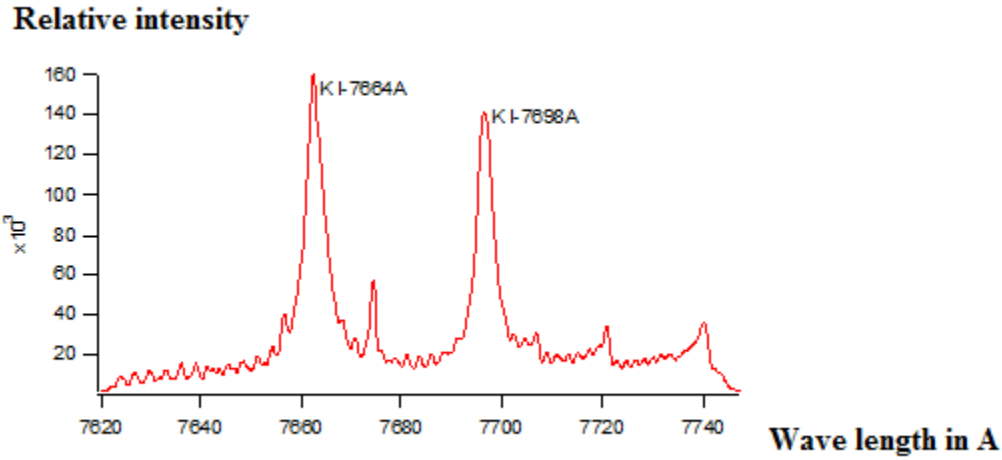
(a)



(b)



(c)



(d)

Figure 4.1: (a)-(d) LIBS spectra of coffee

The variations in term of relative intensity of the emission lines of some elements in our coffee samples are gathered and shown in figure 4.2 using histogram. From the histogram we can observe that in our coffee samples the amount of magnesium, calcium and potassium is relatively higher than the amount of copper, aluminum and sodium. Moreover in our coffee samples we have identified zinc, manganese and iron but in small amount.

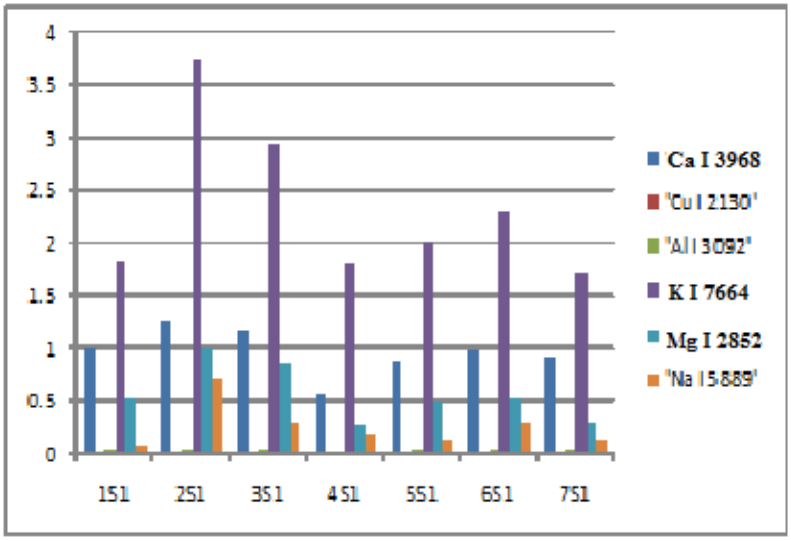


Figure 4.2: Elemental variations in terms of integrated intensity of emission lines.

A couple of researches have done elemental analysis of Ethiopian coffee. The first was done by Frezer Kassa and A.V.Gholap (Frezer Kassa 2013).On their work they analyzed four Ethiopian coffee samples (Washed Lekempti, Washed Yirgacheffe, Washed Sidama and Unwashed Harar) to find the concentrations of five elements (K, Mg, Ca, P and S). The second was done by Abera Gure, B. S. Chandravanshi and Taddese Wondimu (AberaGure 2006). They analyzed five Ethiopian coffee samples from Wollega, Sidamo, Bench Maji, Harar and Kafa to find the Concentrations of metals; (Ca, Cd, Cr, Co, Cu, Fe, K, Mg, Mn, Ni, Pb and Zn) by flame atomic absorption spectrometer FAAS technique (table 4.1). In both cases the results they have got in terms of the variation of the amounts of elements are similar to what we have found in our researches.

Table 4.1: Concentration of elements in coffee analyzed by FASS technique.

Elements in coffee	Wolga coffee mg/g	Sidama coffee mg/g	Harrar coffee mg/g
Ca	0.90 ± 0.01	0.880 ± 0.01	0.71± 0.05
K	14.5 ± 0.30	14.1 ± 0.70	13.9 ± 0.90
Mg	1.67 ± 0.02	1.67 ± 0.02	1.67 ± 0.03

Normalization of the emission spectra to the most intense emission line deletes the differences between spectra due to different amounts of sample or pathlength variation (J.A. Mike, 1995). Part of the data table and data normalized by carbon line (CI-2428 nm) of the integrated intensities are displayed in table 4.2 and table 4.3 respectively.

Table 4.2: part of data table of the emission lines of elements in coffe

Samples	'Cu I 2130'	'Cu I 2140'	'C I 2478'	'Mg II 2790'	'Mg II 2795'	'Mg II 2797'	'Mg II2802'	'Mg I 2852'	'Al I3082'
1S1	**6291**	**4571**	**371200**	**52842**	**812842**	**99642**	**631381**	**384000**	**3079**
1S2	6271	4555	371100	52672	812600	99425	619880	387280	3110
1S3	6250	4590	371300	52672	812710	99425	619880	387280	3093
2S1	3713	2410	216751	29458	498110	56369	362635	273458	1721
2S2	3632	2390	216730	29431	498036	56331	362601	273440	1706
2S3	3674	2402	216721	29449	498080	56357	362620	273421	1692
3S1	4798	3216	267061	22390	461000	40962	342000	318064	1301
3S2	4809	3221	267000	22378	461000	40950	341988	318000	1290
3S3	4815	3227	266982	22401	461060	40971	342030	317984	1309
4S1	4945	3239	340121	61146	782104	122000	593121	194109	884
4S2	5041	3262	339889	61204	781901	122106	593000	194000	812
4S3	4980	3262	340000	61069	782000	121923	592985	193978	830
5S1	6384	4518	313894	72501	842141	127112	620142	278000	1667
5S2	6368	4489	314000	72605	841856	126889	619886	278128	1623
5S3	6398	4534	314123	72552	842000	127000	620000	277869	1645

Table 4.3: part of normalized data table by carbon line (CI-2428 nm) of the emission lines of elements in coffee.

sample	'Cu I 2130'	'Cu I 2140'	'Mg II 2790'	'Mg II 2795'	'Mg II 2797'	'Mg II2802'	'Mg I 2852'
1S1	0.016947737	0.012314116	0.142354526	2.189768319	0.268432112	1.700918642	1.034482759
1S2	0.01689841	0.01227432	0.141934788	2.189706279	0.267919698	1.670385341	1.043600108
1S3	0.01683275	0.012361971	0.141858336	2.188823054	0.267775384	1.669485591	1.043037975
2S1	0.017130255	0.011118749	0.135907101	2.298074749	0.260063391	1.673048798	1.261622784
2S2	0.016758178	0.011027546	0.13579569	2.297955982	0.259913256	1.67305403	1.261661976
2S3	0.016952672	0.011083374	0.135884386	2.298254438	0.26004402	1.673211179	1.2616267
3S1	0.017965933	0.012042193	0.083838524	1.726197386	0.153380688	1.280606303	1.19097884
3S2	0.018011236	0.01206367	0.083812734	1.72659176	0.153370787	1.280853933	1.191011236
3S3	0.018034924	0.012086957	0.083904533	1.726932902	0.153459784	1.281097602	1.191031605
4S1	0.014538943	0.009523081	0.179777197	2.299487535	0.358695876	1.743852923	0.570705719
4S2	0.014831313	0.009597251	0.180070552	2.300459856	0.35925258	1.744687236	0.570774576
4S3	0.014647059	0.009594118	0.179614706	2.3	0.358597059	1.744073529	0.570523529
5S1	0.020338076	0.014393394	0.230972876	2.682883394	0.40495199	1.975641459	0.885649296
5S2	0.020280255	0.014296178	0.231226115	2.681070064	0.404105096	1.974159236	0.885757962
5S3	0.020367818	0.014433836	0.230966851	2.680478666	0.404300226	1.973749136	0.884586611

4.3 Characterizations of coffee samples

4.3.1 Steps for using PCA

In PCA we arrange data in a matrix, for example, with samples arranged as a row and the

spectra of the elements arranged as columns from which we will find the covariance matrix.

Covariance provides a measure of the strength of the correlation between two or more sets of random variables. A large (small) value indicates high (low) redundancy. It captures the correlations between all possible pairs of measurements. The correlation values reflect the noise and redundancy in our measurements. The diagonal terms, by assumption, large (small) values correspond to interesting dynamics (or noise).The off-diagonal terms large (small) values correspond to high (low) redundancy. The eigenvectors and eigenvalues of the covariance matrix provide us with information about the patterns in the data. By this process of taking the eigenvectors of the covariance matrix, we have been able to extract the most important lines that characterize the sample. The eigenvector with the highest eigenvalue is the first principal component of the data set. We can decide to ignore the components of lesser significance, without losing much information of the original data. Here is where the notion of data compression and reduced dimensionality comes into PCA. Finally we project the input data onto the main principal components, which forms the representation of our data (Parinya Sanguansat 2012).

The preprocessed data matrix was subjected to PCA in R package in order to have an overview of existing trends and discover the main property variations in the data. First we tried to characterizes our samples by using forty nine atomic and ionic lines of Zn,Cu,Mg,Al,Ca, K, Mn and Na as shown in the figure 4.3.

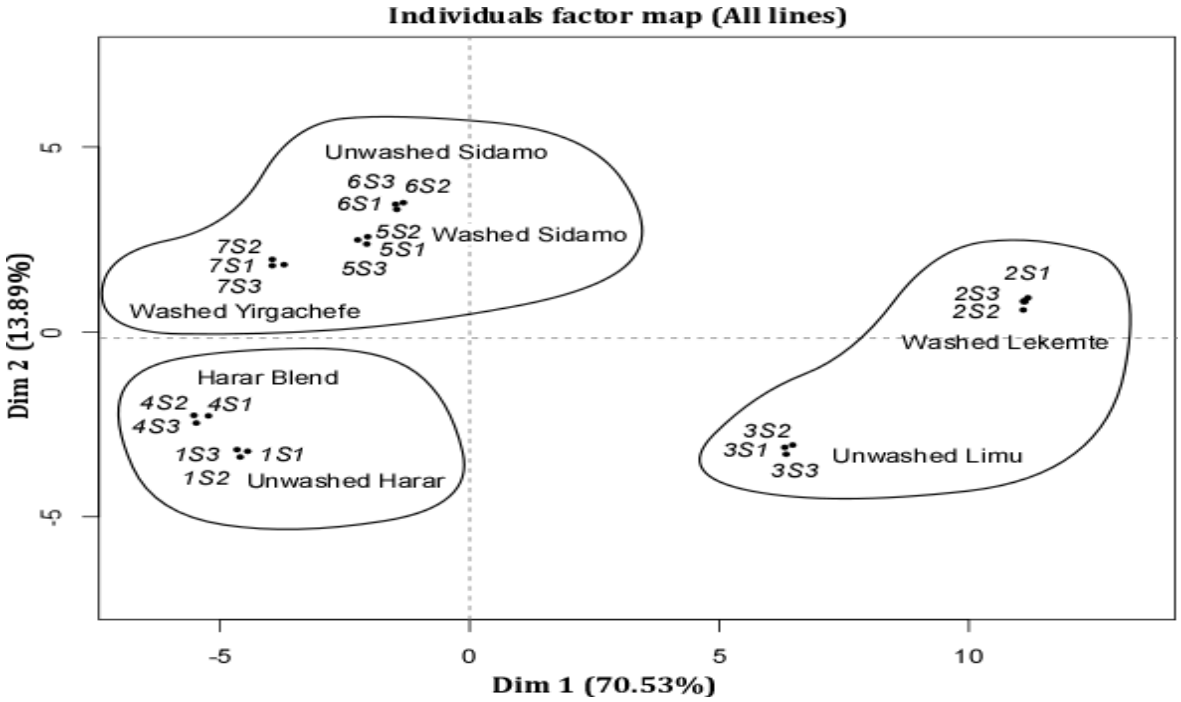


Figure 4.3: characterization of pure coffee samples by using 49 atomic and ionic emission lines

Then by observing the variable factor map shown in figure 4.4 we have selected four lines (Ca I-4226Å, Ca I-3158Å,Mg I-2797Å and Mg I-3832Å) to characterize both pure and mixed coffee samples as well as only the pure coffee samples in the figure 4.5 and figure 4.6 respectively. Finally we have optimized our characterization of coffee samples by choosing only two lines (Ca I- 4226Å and Mg I-2797Å) as indicated in figure 4.7.

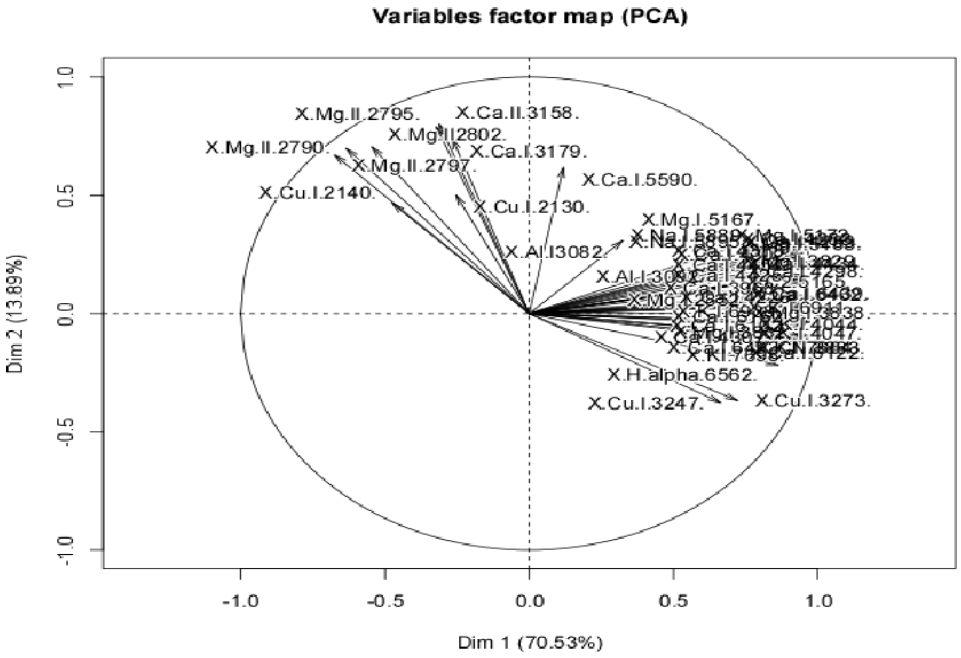


Figure 4.4: variable factor map

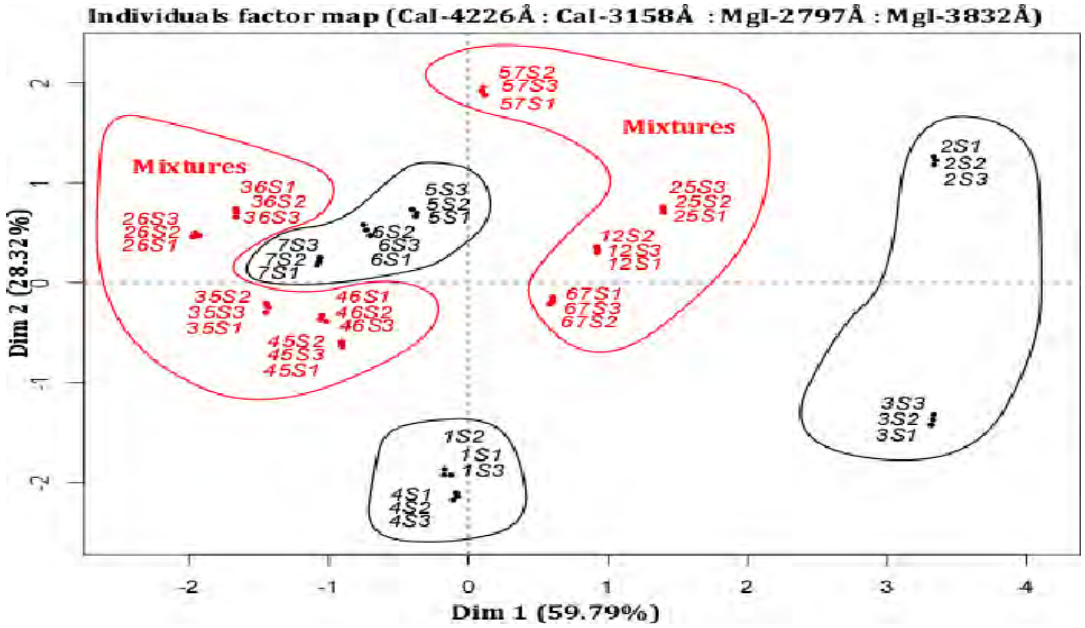


Figure 4.5: characterization of both pure and mixed coffee samples by using only 4 emission lines.

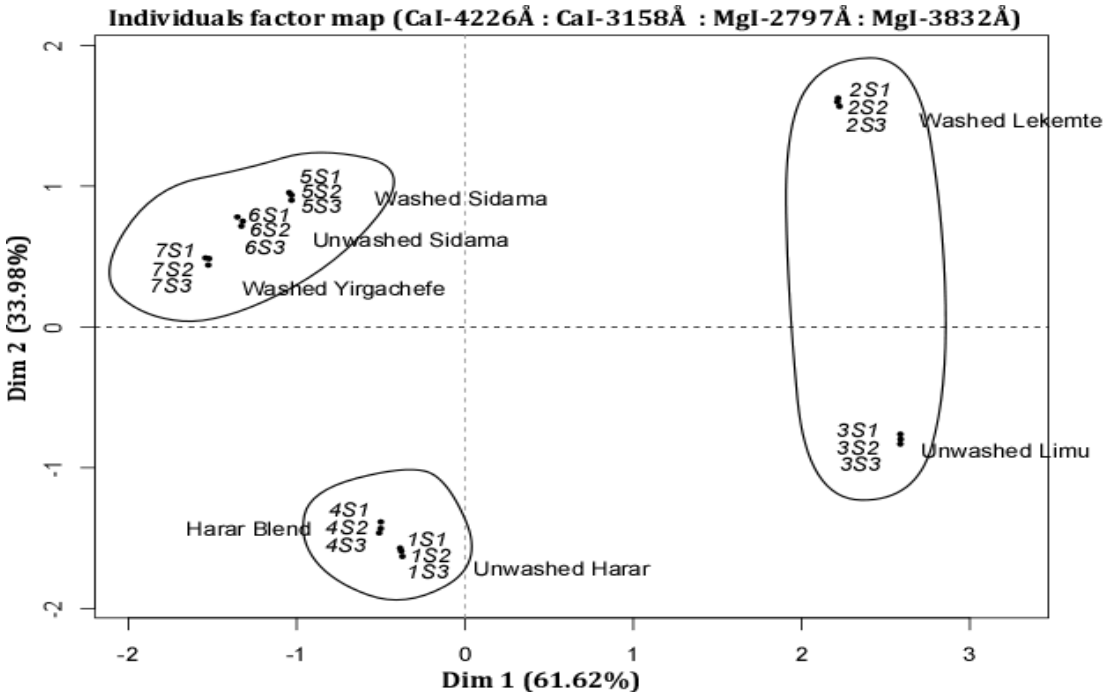


Figure 4.6: characterization of both pure coffee samples by using only 4 emission lines.

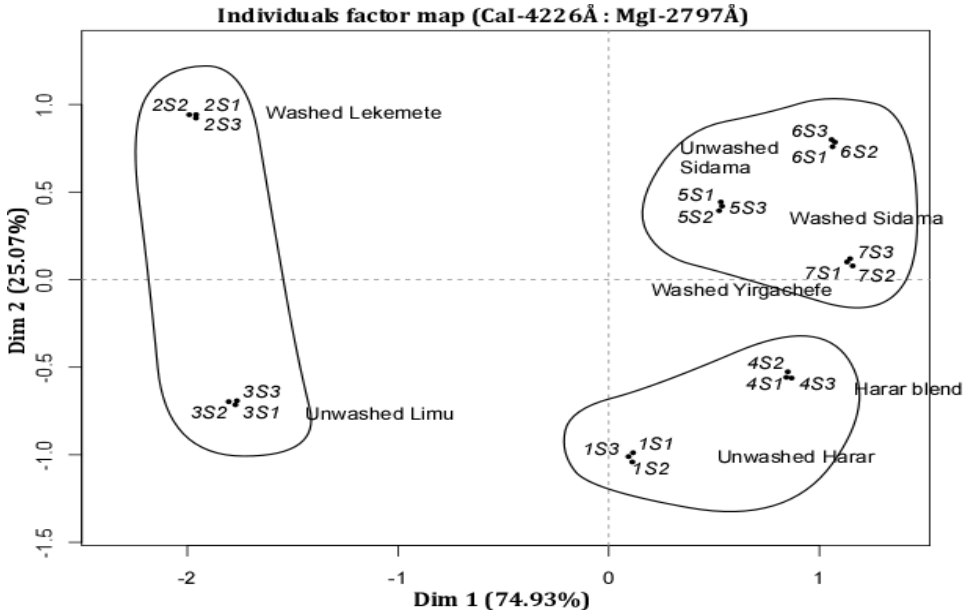


Figure 4.7: Characterizations of pure coffee samples by using two lines only

The scores plots show the coordinates of the spectra in the plane of the two first principal components (PCs), i.e. PC1 (70.53%) and PC 2 (13.89%) which contains 84.42% of the total

variance spectral information. The samples belonging to the same geographical origins tend to cluster together and in almost all cases are separated from the other classes. In the score plots coffee samples from southern region of Ethiopia: washed Sidama (5S1, 5S2, 5S3), unwashed Sidama (6S1, 6S2, 6S3) and washed Yirgachefe (7S1, 7S2, 7S3) clustered together. Coffee samples from eastern regions of Ethiopia: unwashed Harar (1S1, 1S2, 1S3) and Harar blend (4S1, 4S2, 4S3) clustered together and also coffee samples from western region of Ethiopia: washed Lekemte (2S1, 2S2, 2S3) unwashed Limu (3S1, 3S2, 3S3) clustered together. As we mentioned earlier that the quality of Ethiopian coffee is determined by two main factors namely the geographic origin and the post harvest processing. The content of elements in coffee seems to be a good indicator of the coffee authenticity. It can bring the useful information about individual elemental patterns that are distinctive to the origin of growing soils for coffee plants in addition to cultivation and environmental conditions used. This is clearly confirmed from the results obtained in this study. The results clearly indicate that Ethiopian coffee samples can be differentiated by using only calcium and magnesium lines. Moreover the clustering of the three pellets for each sample at the same point shows the repeatability of our experiments.

4.4 Inspection of adulteration of coffee.

Discrimination of coffee samples from their mixtures is important for ensuring reasonable competition and as a means of protecting consumers against deception due to mislabeling. Due to this there is a demand to have a fast and reliable means of measurement allowing discrimination of coffee samples. In our researches we have pointed out that LIBS with appropriate multivariate chemometric method serve the desired purpose. In the figure 4.8, figure 4.9 and figure 4.10 we have displayed the PCA results of sample 3, sample 6 and sample 4with their mixtures respectively.

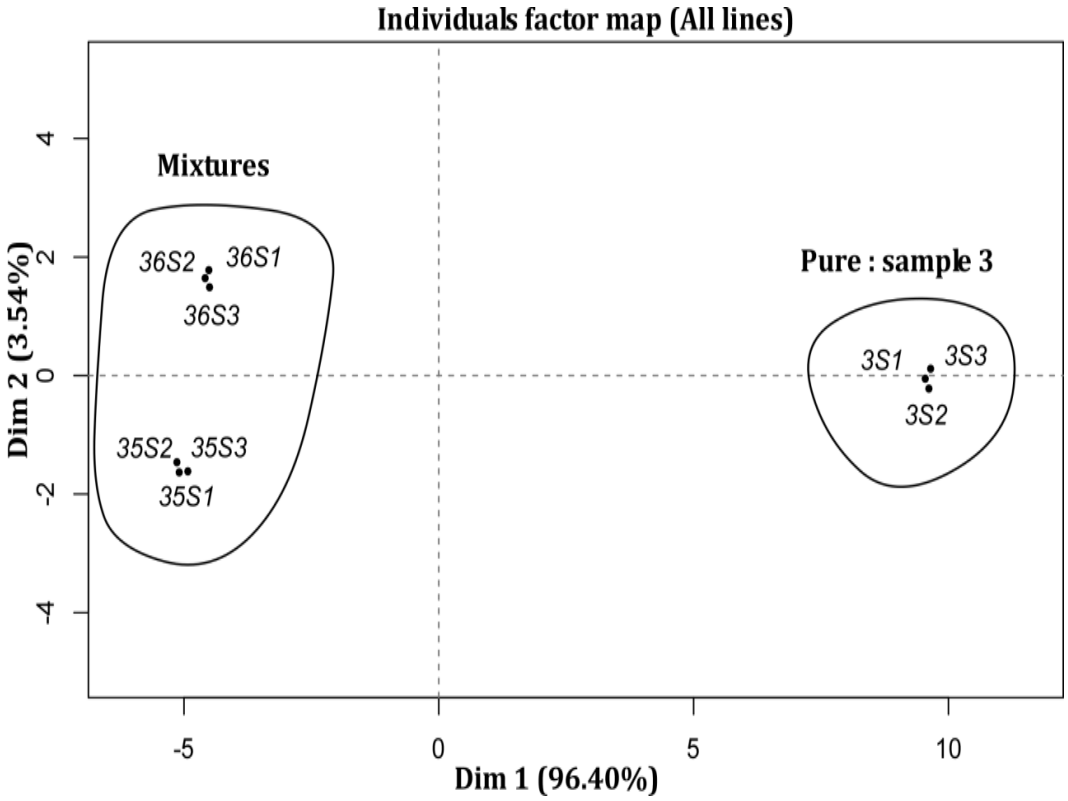


Figure 4.8: discrimination of sample 3 from its mixtures

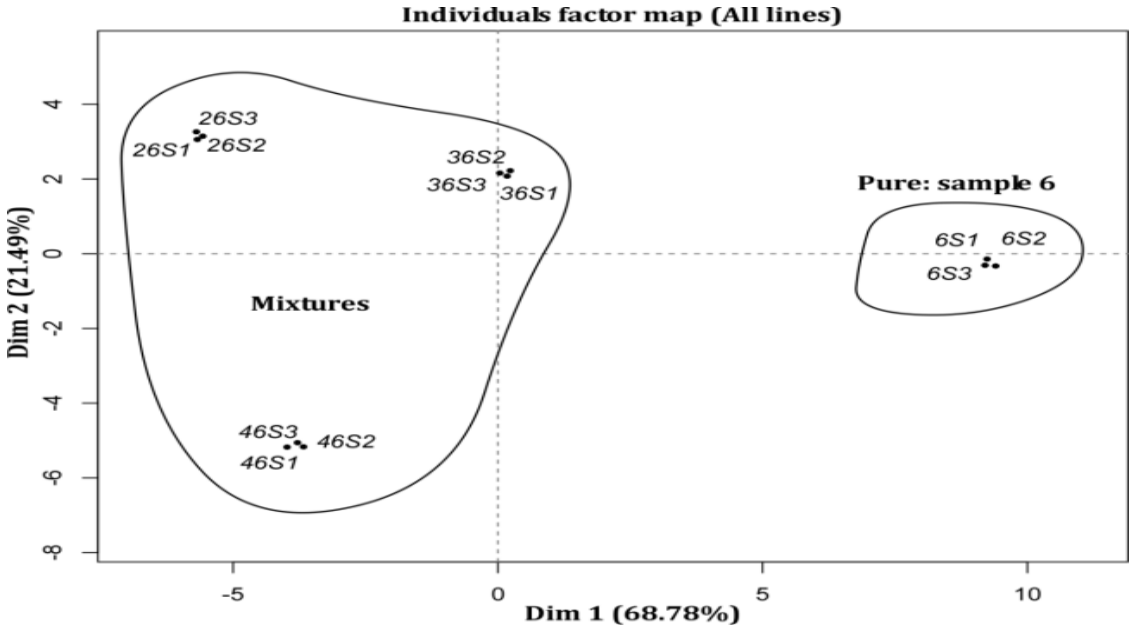


Figure 4.9: discrimination of sample 6 from its mixtures

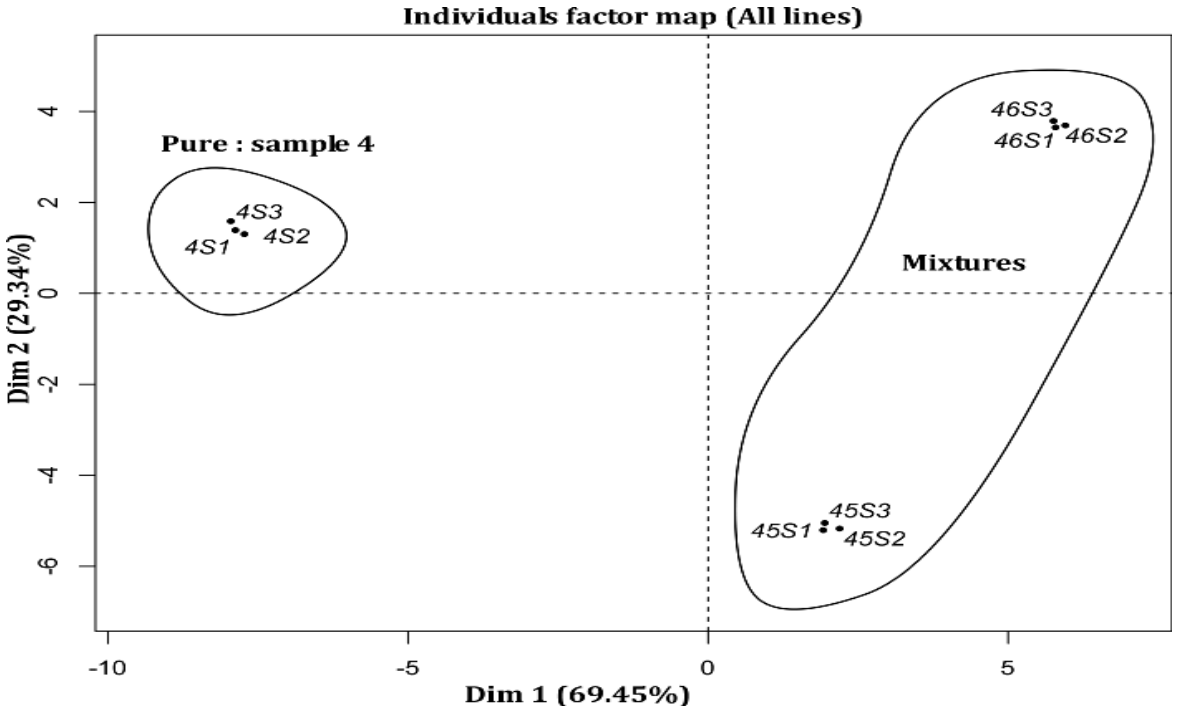


Figure 4.10: discrimination of sample 4 from its mixtures

As we see from PCA score plots in almost all cases the pure samples are separated from the mixtures. According to these results, we can build, based on the elemental emission lines, a fast and efficient technique to detect adulteration of coffee.

PART II

5

Herbal medicines

5.1 Introduction

In this chapter we will discuss about the overview of medicinal plants in Ethiopia, current status of medicinal plants in Ethiopia, quality of herbal medicines and elemental analysis of herbal medicines.

Since ancient times humanity has depended on the diversity of plant resources for food, clothing, shelter, and traditional medicine to cure myriads of ailments. Traditional medicine evolved over centuries, depending on local flora, culture, and religion. Indeed, well into the twentieth century, much of the pharmacopeia of scientific medicine was derived from the herbal lore of native people. This knowledge of plant-based drugs developed gradually and was passed on, thus laying the foundation for many systems of traditional medicine all over the world(Cassileth 1998; Lans 2001; Cragg 2001). Herbal medicine can broadly be classified into a few basic systems:

1. Ayurvedic herbalism (derived from the Sanskrit word *ayurveda*, meaning "the science of life"), which originated in India more than 5000 years ago and was also practiced in neighboring countries such as Sri Lanka.
2. Chinese herbalism, which is a part of traditional oriental medicine.
3. African herbalism.
4. Western herbalism, which originated from Greece and Rome and then spread to Europe and North and South America.

Chinese and Ayurvedic herbalism have developed into highly sophisticated systems of diagnosis and treatment over the centuries. Both have a long and impressive history of effectiveness. Western herbalism today is primarily a system of folk medicine. A European healing tradition, sometimes called the "wise woman" also focuses primarily on herbal healing.

Medicinal plants have played a key role in world health. They are distributed worldwide, but they are most abundant in tropical countries. It is estimated that about 25% of all modern medicines are directly or indirectly derived from higher plants (Ackerknecht 1973; Majno 1975; Farnsworth 1976; Duke 1994; De Smet 1995; Cragg 1997; Shu 1998, Liu 2001; WHO1979-2005).

By definition, a herb is a plant or a part of a plant valued for its medicinal, aromatic, or savory qualities. Herbs can be viewed as biosynthetic chemical laboratories, producing a number of chemical compounds. Herbal medicine or herbalism is the use of herbs or herbal products for their therapeutic or medicinal value. They are also referred to as botanicals, biomedicines, or herbal supplements. Herbal drugs range parts of plants to isolated, purified active constituents. They may come from any part of the plant but are most commonly made from leaves, roots, bark, seeds, and flowers. They are eaten, swallowed, drunk, inhaled, or applied to the skin (Akerele 1993).

Due to poverty and limited access to modern medicine, about four billion people, 80% of the world's population, living in developing countries use herbal medicine as their source of primary health care (Bisset, 1994; Farnsworth, 1985; Mukherjee, 2002; Bodeker, 2005). In these communities, traditional medical practice is often viewed as an integral part of their culture. In comparison with modern medicine, herbal medicines cost less and are more often used to treat chronic diseases

In the West, people are attracted to herbal therapies for many reasons, the most important reason being that, like their ancestors, they believe it will help them live healthier lives. Herbal medicines are often viewed as a balanced and moderate approach to healing. Individuals who use them as home remedies and over-the-counter drugs spend billions of dollars on herbal products.

As such, they represent a substantial proportion of the global drug market (WHO 1990; 1999; 2002; 2005; Akerele 1993; Bisset 1994; Pal 2003; Farnsworth 1985;).

5.2 Medicinal plants in Ethiopia

5.2.1 Introduction

The various literature available show the significant role of medicinal plant in primary health care delivery in Ethiopia where 70% of human and 90% of livestock population depend on traditional medicine again similar to many developing countries particularly that of Sub-Saharan African countries. Those plants are part of the economic commodity for some members of the society which make their livelihood on their collection, trade and medicinal practices by practitioners or healers. It thus has a substantial potential to make contributions to the economic growth and alleviation of poverty in the country. Its proper management protect environment and conserve biodiversity. The traditional health care is deeprooted with oral and written pharmacopoeias. Ethiopian plants have shown very effective contributions for some ailments of human and domestic animals. Such plants include *Phytolacca dodecandra* (Aklilu Lemma, 1965), Many species of *Maytenus* studied by National Cancer Institute, USA see Kupchan et al. 1972 and many species that show antimalarials (Nkunya, M. H. H., 1992).

Medicinal plants and knowledge of their use provide a vital contribution to human and livestock health care needs throughout Ethiopia. The research made so far on Ethiopian medicinal plants has been mostly of producing inventories and checklists, only very few have been touched by modern research where their principal component has been analyzed and defined.

The bulk of the plant matter used for medicinal purposes is collected from natural vegetation stocks that are shrinking with degraded environment and to substantial reduction or dwindling of species of medicinal plants. According to Ensermu Kelbessa et al. (1992) and Edwards (2001),

habitat and species are being lost rapidly as a result of the combined effects of environmental degradation, agricultural expansion, deforestation and over harvesting of species and this is further enhanced by human and livestock population increase thus hastening the overall rural livelihood impoverishment and loss of the biological diversity and indigenous knowledge which is also of global concern since some of this are endemic to Ethiopia. A full scale plan to conserve, develop and effectively utilize this resource needs investment commitments by government agencies, the private sector, and various global foreign aids for development. However, before such investments and support are realized, a clear indication of the resource condition and its economic values must be worked out. This needs a critical overview of medicinal plants in Ethiopia, their demands, trade, and economic benefits. Such an overview has to come up with a formulation of the strength, weakness and opportunities in the medicinal plant sector to forward conclusions and recommendation.

5.2.2 Overview of medicinal plants in Ethiopia

Ethiopia is believed to be home for about 6,500 species of higher plants with approximately 12% endemism, and hence one of the six plant biodiversity rich countries of Africa (UNEP, 1995). The diversity is also considerable in the lower plants but exact estimate of these have to be made. The genetic diversity contained in the various biotic make up is also high thus making the country a critical diversity hot spot for plants.

As one of the 12th Vavilovian centers of origin/ diversity for domesticated crops and their wild relatives, it is home of many endemic crops and genetic stocks (Vavilov, 1951; Harlan, 1969; Endashaw Bekele, 1978).

Ethiopia has a significant portion of two of the world's 25 biodiversity rich areas hot spot i.e. the eastern Afromontane Biodiversity Hotspot and the Horn of Africa-Biodiversity Hot Spot

(Conservation International at www. biodiversityhotspots.org). These hotspots house a lot of the useful w ild bi odiversity, pa rticularly t hat of m edicinal pl ants. T he bi odiversity r ichness of Ethiopia was known since 5000 years ago when ancient Egyptians Greeks and Romans used it as a s ource o f uni que c ommodities l ike F rankincense, M yrrh a nd ot her pl ant pr oducts, w hich a re also used for medicine preparation (Thulin, 2004).

Most Ethiopian traditional medicinal knowledge is kept in strict secrecy; however, it is dynamic in that the practitioners make every effort to widen their scope by reciprocal exchange of limited information w ith e ach o ther or t hrough reading the t raditional pha rmacopeias (Dawit A bebe, 1986). Dawit Abebe (1986) gives three treatment features of Ethiopian traditional medicines i.e. curative, prophylactic and preventive. Sometimes, the treatment could have a curative as well as a prophylactic effect and it is occasionally claimed that the prophylaxis could even be genetically fixed and can protect the offspring. Preventive remedies are usually prepared as ornamental, to be bor ne b y t he pa tients a gainst e vil s pirits or ps ychosomatic di sorders. O ther t herapies of preventive na ture a re employed against s nake b ites, in testinal w orms, a nd mis carriages. Regulatory drugs are a lso c ommonly us ed t o correct t he t ime and t he amount of f low o f t he menstruation c ycle of women. R ejuvinative a nd r estorative r emedies a re a lso e mployed t o counter the effect of aging, and to overcome impotence, malnutrition, infertility etc. Traditional medicine is an integral part of the local culture and is a major public health system; what we call modern medicine is an offshoot of traditional medicine.

1000 i dentified m edicinal pl ant s pecies a re r eported i n t he E thiopian F lora, how ever, m any others a re not yet i dentified. A bout 300 of t hese s pecies a re f requently mentioned i n m any sources. (Jansen, 1981) a sserts t hat E thiopia ha s r ich m edicinal pl ant l ore a nd poi nts out t hat almost a ll pl ants of t he E thiopian f lora a re us ed s omewhere s omehow m edicinally. O ther

workers on the other hand estimated about 60% of the flora to be medicinal, and most sources give about 10% of the vascular flora to be medicinal. The list cover plants that are widely used by the local communities in lowlands and highlands for treating human ailments and some of them for livestock ailments as well as for prevention of pests and vectors.

Study on the Bale Mountains National Park in the South East Ethiopia revealed that the area, as much as it is a biodiversity hotspot, also turned out to be a medicinal plant hotspot with 337 identified medicinal species of which 24 are endemic (National Herbarium, 2004; Ermias Lulekal, 2005; Haile Yineger, 2005). The species comprised of 283 used as human medicine, 47 used as livestock medicine and 76 species used for both human and livestock by the community healers, harvesters, traders and users. This work further suggested spots that could be considered medicinal plant micro - hotspots within the Bale Mountain area.

Many species of Ethiopian medicinal plants have a long history of use as remedies. The traditional medicinal systems in different parts of the world have some distinctive features. Chinese traditional herbal medicine, the Indian Ayurvedic medicine, the Japanese traditional medicine system and the African system are recognized among others. The Ethiopian traditional medical system is mainly a subcategory of the African traditional medical system with some influence from Egypt and Greece and has its own characteristic features. Ethiopian traditional life is painted with the hallmark of widespread use of traditional medicinal plants with various levels of sophistication within the indigenous medicinal lore. It is blended with religious thinking and various beliefs and need further investigation. The basic categories of practitioners also are difficult to define.

5.2.3 Current states of traditional medicines in Ethiopia

Ethiopia has policies and strategies that support the development and utilization of plant resources in a sustainable manner. The policies are reflected under various sectors including environmental protection, development of the natural resources and diversification of the domestic and export commodities. Medicinal plants fit in the development activities that support public efforts in meeting livelihood requirements. There are few institutions concerned with the medicinal plants and assisted through government budgetary support. The Ethiopian Health and Nutrition Institute receives annual budget of about ETB 1.1 million while a Department at IBC concerned with medicinal plant conservation gets ETB 100, 000 per annum. The recent ongoing support made through the project funded by the World Bank namely the conservation and sustainable use of medicinal plants project has an annual budget of ETB 5.9 million per year during the project life. Such a support indicates the importance Ethiopia has given to the sector. The health sector strategy of Ethiopia declares that structural, functional traditional medicine into the official health care system is advantageous for improving the health coverage in the country. However suitable institutional mechanisms and detailed implementation strategies and action (Ministry of Health, 1995) plans has to be put in place.

5.3 Quality of Herbal Medicines

Typically, there is no one single herb that is recommended for a given health disorder; and there is no one single health disorder linked with just one single herb. Herbal products often contain a variety of bio-chemicals found naturally in the plants and many different bio-chemicals contribute to a plant's medicinal benefit. Chemicals known to have medicinal benefits are referred to as "active ingredients," and their presence depends on the plant species, the way the herb is prepared, the time and season of harvest, the type of soil, etc. Most herbal products

contain plant parts or plant materials in the crude or processed state as active ingredients and certain recipients, such as solvents, diluents, or preservatives. In most cases, the active principles responsible for their pharmacological action are unknown. The general perception that herbal drugs are very safe and free from side effects is not true. Herbs can produce undesirable side effects and can be toxic.(Bisset 1994; Pal 2003).

Herbal medicines are very different from well-defined synthetic drugs. For example, the availability and quality of the raw materials are frequently problematic; the active principles are frequently unknown; and standardization, stability, and quality control are feasible but not easy.

In most countries herbal products are launched into the market without proper scientific evaluation, and without any mandatory safety and toxicological studies. There is no effective machinery to regulate manufacturing practices and quality standards. Consumers can buy herbal products without a prescription and one might not recognize the potential hazards in an inferior product. A well-defined and stable composition of the drug is therefore one of the most important prerequisites for the production of a quality drug. Given the nature of products of plant origin, which by definition are never constant and are dependent on and influenced by many factors, quality control plays a significant role for the industry to thrive and be successful.

A drug is defined as being safe if it causes no known or potential harm to users. Data will be required on the following: Acute toxicity, long-term toxicity. Data may also be necessary on the following: Organ-targeted toxicity, immune-toxicity, Embryo/fetal and prenatal toxicity, Mutagenicity/genotoxicity, Carcinogenicity (Bisset 1994; Farnsworth 1998).

5.4 Elemental analysis of herbal medicines

Most studies on such medicinal plants pertain to their organic contents like essential oils, glycosides, vitamins, alkaloids and other active components and their pharmacological and

therapeutic effects. Besides several organic compounds, it is now well established that many trace elements play a vital role in general well-being as well as in the cure of diseases (Underwood, 1977; Prasad, 1993).

Many elements in trace amount in human body play an essential role in metabolic process (Dell and Sunde, 1997). Therefore, identification of elements in medicinal plant and food products are vital. Minerals are involved in structural components of human tissues, resources of acid-base balance and maintain the body fluids, transport of gases and muscle contractions (Omaye and Reddy, 1962).

Mineral deficiencies have manifested in forms of different disease conditions as goiter, rickets, and one form of metabolic dysfunction or the other. Minerals are divided into two groups: major minerals and trace minerals. The body needs larger amounts of major minerals than trace minerals, although trace minerals can be just as important for good health (Frank W. Cawood, 1997; Chaney, 2006; Crook,2006).The major minerals include calcium, chloride, phosphorus, potassium, sodium, sulphur, and magnesium, while the trace minerals include iodine, iron, zinc, selenium, fluoride, chromium, copper, molybdenum, and manganese (Frank W. Cawood, 1997; Chaney, 2006; Crook, 2006).

Mineral contents of various medicinal plants were evaluated and correlated with their therapeutic action by numerous workers (Sahito, 2003; Pirzada, 2005; Januja, 1990; Sailey, 1994; Singh AK, 2011). Atomic absorption spectroscopy (AAS) (Herber and Stoeppler, 1994), energy dispersive X-ray fluorescence spectrometry (EDXRF) (Joseph, 1999), electro thermal atomic absorption spectrometry (ETAAS) (Scancar, 2000), inductively coupled plasma-atomic emission spectrometry (ICPAES), inductively coupled plasma mass spectroscopy(ICPMS) (Chan and Lo, 2003) are some of the techniques employed for the elemental analysis of medicinal plants.

In cases of ETAAS, ICPAES and ICPMS analytical methods medicinal plant samples require to be appropriately prepared before measurements by the digestion and the mineralization of their organic matrix.

As we have displayed in chapter seven of this study our results are in good agreements with the other analytical methods used.

6

Experimental methods for herbal medicines

6.1 Description of herbal medicines studied

In this section we will briefly give the descriptions of the use of the medicinal plants studied in our r esearches r elated to E thiopian t raditional h erbal m edicine p ractices. Ten E thiopian medicinal plants and their two mixtures are analyzed by using LIBS technique. The names of the samples are shown in the table 6.1 below

Table 6.1: The samples studied with the chosen nomenclature

No	scientific name	Amharic name
Sample 1	*Ocimum lamifolium*	Demakese
Sample 2	*Zingiber officinale*	Zingibil
Sample 3	*Moringa stenopetala packed*	Shiferaw packed
Sample 4	*Echinops kebericho*	Kebercho
Sample 5	*Foeniculum vulgare*	Ensilal
Sample 6	Artemisia afra	Ariti
Sample 7	*Lepidium sativum*	Fieto
Sample 8	*Moringa stenopetala natural*	Shiferaw natural
Sample 9	Coriandrum sativum	Dimbelal
Sample 10	Ruta chalepensis	Tenadam
Sample11	mixture of *Ocimum la mifolium* and *Foeniculum vulgare*	Mixture of Demakese and Ensilal
Sample 12	mixture of *coriandrum s ativum* and *zingiber officinale*	mixture o f Dinbelal and Zingibil

***Ocimum lamifolium* (Demakese)**

Used to treat coughs and colds, the fresh leaves are squeezed and the juice sniffed. The juice can also be used as an eye rinse for eye infection. Also used for *mich*, an infection of fever with headache and mouth blisters (Gedif, T. and Hahn, H.J., 2003).

Figure 6.1: *Ocimum lamifolium* (Demakese)

***Zingiber officinale* (Zingibil)**

Figure 6.2: *Zingiber officinale* (Zingibil)

The rhizome (root) of ginger is popularly used in Ethiopia for stomachache and respiratory problems. It is chewed or masticated with 'feto' (*Lepidium sativum)* for stomach disorders. It is also popularly used for its carminative (relieves gas) and anti-nausea activities. Ginger is equally popular in western herbal medicine and there has been extensive investigation of the rhizome and its constituents. *Zingiber officinale* has demonstrated anti-inflammatory effects, as well as anti-platelet, antioxidant, antitumour, antirhinoviral and antihepatoxic activities (preventing damage to the liver) (Wohlmuth, 1999).

***Echinops kebericho* (Kebercho)**

Figure 6.3: Echinops Kebericho.

Endemic to Ethiopia, *Echinops kebericho*, is used for fever and as a taenicidal herb (to expell tapeworm). The smoke from burning the plant is inhaled to relieve headache. The root is burned for smoke to ward off mosquitoes and as a snake repellant in the house. The smoke is inhaled to fight typhus and fever, and is known to be used as a fumigant, mainly after childbirth. The root is also chewed to alleviate stomach ache (Demissew, S., 1993).

Foeniculum vulgare **(Ensilal)**

Figure 6.4: *Foeniculum vulgare* (Ensilal)

In Ethiopia, the boiled or roasted roots of Ensilal or Fennel are traditionally used to treat gonorrohoea, digestive disorders and infant colic. The juice of the fresh or dried leaves is used to stem nosebleeds and the plant is also known for its anti-fertility properties. Studies record the traditional use of an oral application of the fresh Fennel leaf as an anti-fertility remedy (Desta, 1995).

Western herbalists are familiar with the use of Fennel seed as a carminative and digestive; and evidence from randomized, double-blind, placebo controlled trials suggest that Fennel is effective in reducing infantile colic (Natural Standard, 2010). Clinical trials also support the use of Fennel in combination with other herbs for dyspeptic conditions of the upper GIT, including pain, nausea, belching and heartburn; chronic non-specific colitis, diarrhoea or constipation (Getahun, A., 1976).

***Artemisia afra* (Ariti)**

Figure 6.5: *Artemisia afra* (Ariti)

Artemisia afra called Ariti in Ethiopia, the juice of the crushed leaves of this plant is mixed with water or honey and administered orally to address stomach pain in Ethiopian traditional medicine practice.

The essential oil of Artemisia Afra has antimicrobial properties. In South Africa, it is one of the most popular and commonly used herbal medicines for treating various ailments; from coughs and colds to malaria and diabetes (Getahun A., 1976).

Moringa *stenopetala (shiferaw)*

Figure 6.6: Moringa *stenopetala (shiferaw)*

Moringa tree has both nutritional and medicinal values. The leaves, flowers, and green pods of *M.stenopetala* are eaten as a staple vegetable and are rich in proteins and Ca, Fe, and P (ICRAF, 2006). *Moringa stenopetala* is a favorite and main component of the daily meal of the Konso, Gamo, and Gofa people in southern Ethiopia (Endeshaw, 2003; Personal observation). Moringa leaves contain seven times the vitamin C of oranges, four times the vitamin A of carrots, four times the calcium of milk, three times the potassium of bananas, and two times the protein of yoghurt (Mathur, 2005) . M oreover, M oringa l eaves c ontain a ll t he e ssential a mino a cids (Mathur, 2005; M elesse e t a l., 2009; S teinmüller e t a l., 2002) a nd vi tamins A a nd C a mong others (Mathur, 2005; Steinmüller et al., 2002). Raw leaves of *M. stenopetala* contain 9% crude protein on a dr y m atter basis (Abuye et a l., 2003) a nd a hi gher pe rcentage of c arbohydrates, crude fiber, and calcium (Abuye et al., 2003). V itamins are present at nutritionally significant levels av eraging 28 mg/100 g of vitamin C and 160 µg/100 g of beta-carotene (Abuye et al ., 2003). M oringa is the richest source of beta-carotene (vitamin A) and provides other important micronutrients (Mathur, 2005). M inerals such as K, Fe, Zn, P, and Ca also exist in significant concentrations with average values of 3.08 mg/100 g iron and 792.8 mg/100 g Ca (Abuye et al.,

2003). Reports indicated that given the high vitamin content, *M. stenopetala* leaves could be used to reduce child and maternal mortality rates in the country by 30-50% (Anon, 2003).

Coriandrum sativum (Dimbelal)

Figure 6.7: *Coriandrum sativum* (Dimbelal)

Local application of coriander seeds alleviates swelling and pains. Paste of green coriander has very good action on headache caused by pitta. Externally, powdered green coriander alleviates burning sensation and pain in diseases like inflammation caused by pitta, erysipelas and lymphadenopathy. Decoction of green coriander is useful in stomatitis. In epistaxis, nasal drops of green coriander act as a haemostat and thus stop bleeding. In conjuctivitis, either juice or decoction of green coriander is put in eyes. The seeds were included in a host of prescriptions for fever, diarrhea, vomiting, indigestion as in stomach and carminative.

Fresh juice of leaves is used as a gargle in sore throat and stomatitis. Paste is applied over swellings and boils; also over cervical adenitis. The paste is prepared by pounding green leaves with barley flour. The paste of dry fruits is applied over forehead and temples during headache. For cooling effect on the mind and for including sleep, fresh juice of the leaves, mixed with sugar, is given. It is also given in biliousness, intestinal irritations, heartburn, thirst and nausea (H. Fassil, 1996).

Lepidium sativum (Fieto)

Figure 6.8: *Lepidium sativum (Fieto)*

Scientific investigations show that, Ethiopia is the origin of *Lepidium sativum (Fieto)* and it is distributed in various areas from Ethiopia. Studies revealed that for both control and diabetic subjects meal with *Lepidium sativum* seeds lowered the glycemic response as compared to the meal without *Lepidium sativum* seeds. They also reported that diabetic subjects showed higher reduction in glycemic response compared to healthy subjects. In long term (21 days) administration of diabetics with *Lepidium sativum* seeds (15 gm/day) 9 out of 11 subjects showed reduction in the levels of blood glucose from 10.2 mM/L to 8.3 mM/L at the end of the study period. Results of this investigation indicate that *Lepidium sativum* seeds have a potential of activity as hypoglycemic activity. Seeds have been shown to reduce the symptoms of asthma and improve lung function in asthmatics, and the seed mucilage is used as a substitute for gum arabic and tragacanth. Some use it in the belief that it can cure asthma, bronchitis bleeding piles. Some use *Lepidium sativum* seeds for indigestion and constipation (Qudiha Pankaj, 1998).

***Ruta chalepensis* (Tenadam)**

Figure 6.9: *Ruta chalepensis* (Tenadam)

Ruta chalepensis is cultivated in several countries in tropical Africa where it is used for cooking and medicinal purposes. The medicinal and culinary properties are attributed to the presence of essential oils which are contained in all parts of the plant. The tops of the fresh shoots are the most active and should be gathered before the plant flowers. A fruit poultice is applied to swellings. In Ethiopia *Ruta chalepensis* is an important medicinal plant. An aqueous-alcoholic extract of the leaves is drunk as an anti-implantation and uterotonic medicine. A decoction of the pulverized fruits in milk is taken to treat diarrhoea. A root decoction in an alcoholic drink, with hot peppers, is taken to treat influenza. Plant sap is taken to treat stomach-ache. A leaf decoction with tea is taken to treat headache, fever and common cold. In southern Africa, the oil obtained from the aerial parts is applied externally as a rub to treat stomach-ache, colic, hysteria, epilepsy and is taken orally as an anthelmintic. A decoction of the whole plant in high doses is taken to ease childbirth. A leaf decoction of *Ruta chalepensis* or *Ruta graveolens* is taken for the treatment of typhoid and scarlet fever, whereas the leaf juice is given to children suffering from convulsions, fits, jaundice and diarrhea. The crushed leaves are externally applied to treat

toothache and earache. A maceration of the leaves is taken to treat cardiac and respiratory diseases, rheumatism, gout and hypertension. Leaves are taken in tea or chewed to treat stomach-ache and headache (Toserkani et al., 2011).

6.2 Sample preparation of herbal medicines

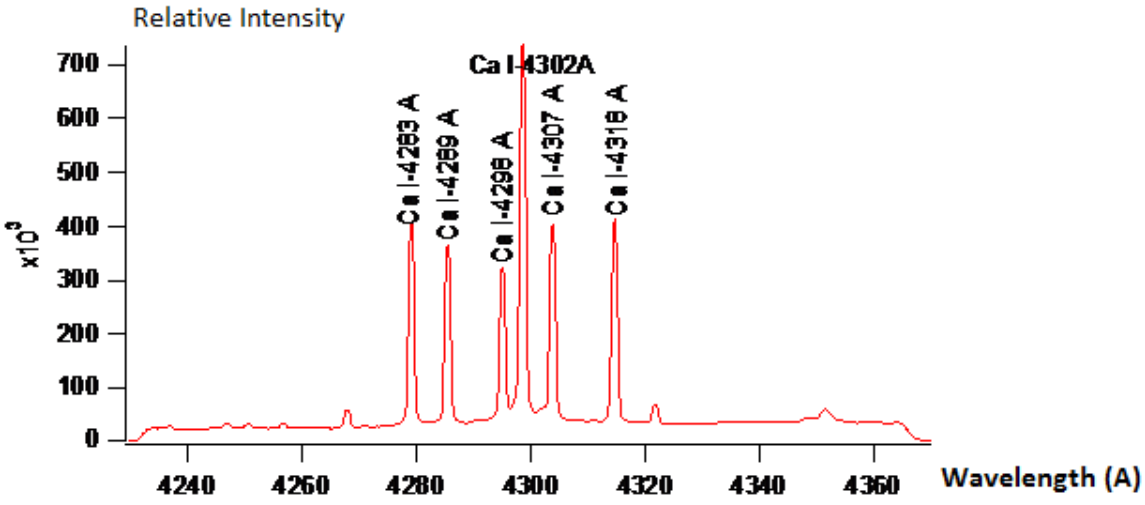
Ten commonly used herbal medicines purchased from local market places and authenticated by the Ethiopian bio-diversity institute were used as samples for analysis. The plant samples were made up of the leaves, stem and roots. The samples were gently and thoroughly washed with distilled water to avoid contamination. They were then dried at room temperatures in the range of 20°-26°C and then grounded into fine powder with grain size ~20 μm. About 300 mg of each sample were pelletized using a hydraulic press with a pressure around 2 tons/cm^2 to produce an intermediate thick pellet samples. We also prepared two samples by mixing two types of herbal medicines in equal amounts 150g each to see the capability of LIBS for monitoring the change in elemental composition of mixtures. For each sample three pellets were prepared to check the repeatability of the experiments.

7

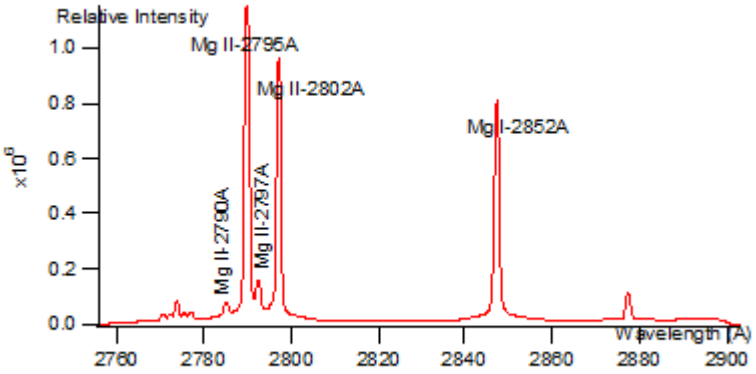
Results and discussions of Herbal medicines

7.1 Analysis of mineral elements in herbal medicines

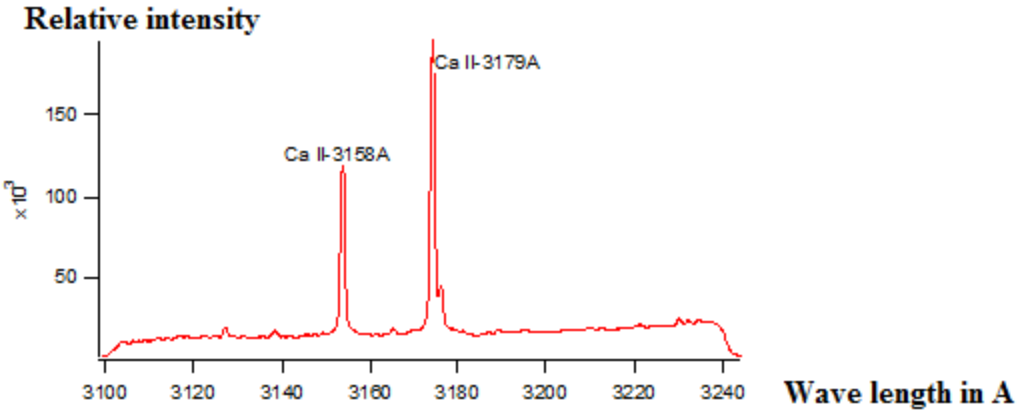
The LIBS spectra of herbal medicinal samples were collected in wavelength range 200-800 nm in a ir a tmosphere. S pectra a cquired b y LIBS a re pr ocessed us ing Igor s oftware. W e ha ve displayed part of the spectra in figure 7.1



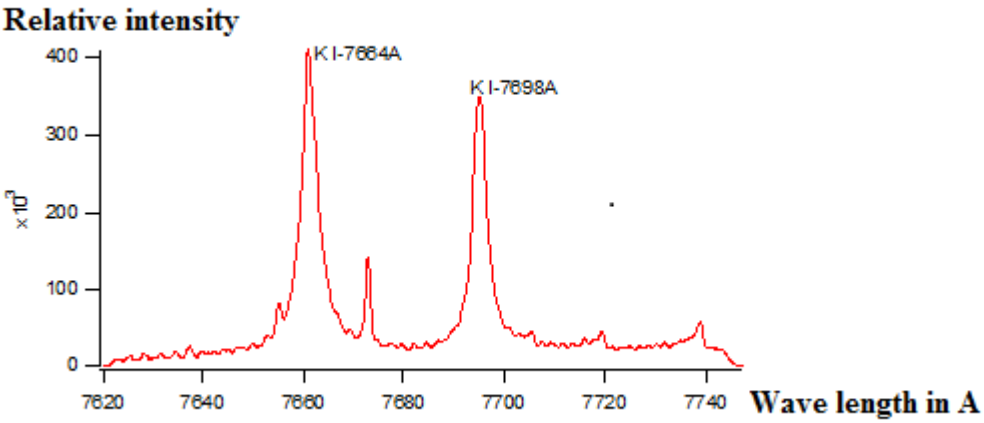
(a)


Relative Intensity
Mg II-2795A
Mg II-2802A
Mg II-2852A
Mg II-2790A
Mg II-2797A
x10^6
1.0
0.8
0.6
0.4
0.2
0.0
2760
2780
2800
2820
2840
2860
2880
2900
Wavelength (A)

(b)


Relative intensity
Ca II-3179A
Ca II-3158A
x10^3
150
100
50
3100
3120
3140
3160
3180
3200
3220
3240
Wave length in A

(c)



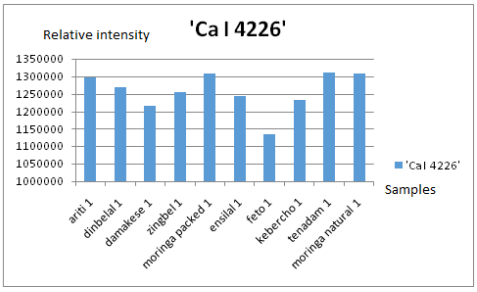
(d)

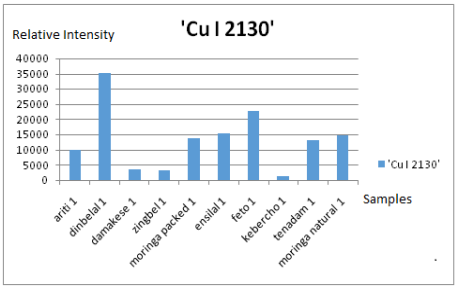
Figure 7.1: (a)-(b) LIBS spectra of *Moringa stenopetala*.

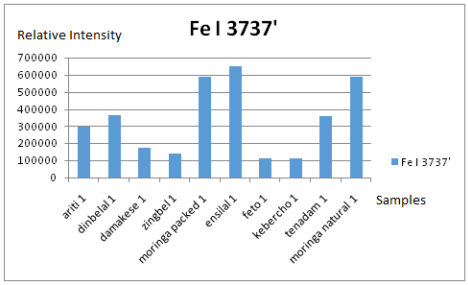
Comparing figure 4.1 and 7.1 we can clearly see that in terms of the integrated intensity the amount of mineral elements in herbal medicines is much larger than the amount in coffee; in fact in this case the amount of calcium in herbal medicines is 15 times larger than the amount of calcium in coffee and also the amount of magnesium in herbal medicines is 3 times larger than the amount of magnesium in coffee. Generally herbal medicines are richer in mineral elements compared to coffee.

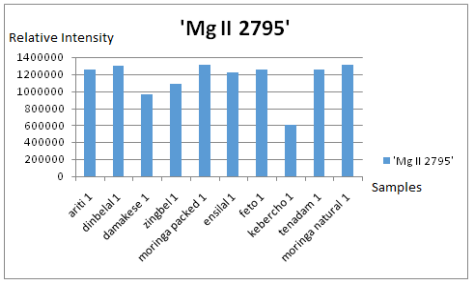
7.2 Comparison of mineral elements in herbal medicines

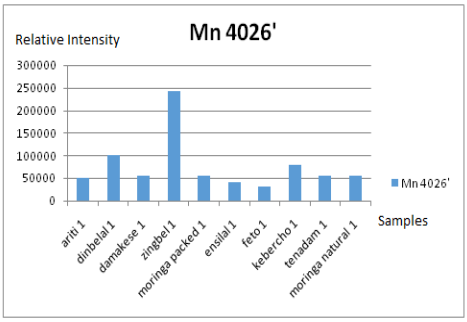
The comparisons of mineral elements in terms of relative intensity of the emission lines are shown in figure 7.2 using histogram. From the histogram we can observe that the amounts of mineral elements were found to vary in different herbal medicines. It is also observed that in our herbal medicine samples the amount of calcium, magnesium and sodium are relatively higher than the amount of iron, potassium, manganese and phosphorus. Moreover in our samples we have identified zinc and copper but in small amounts.

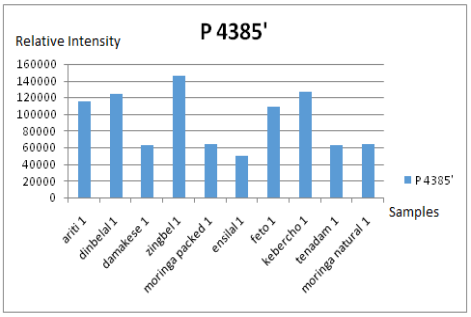


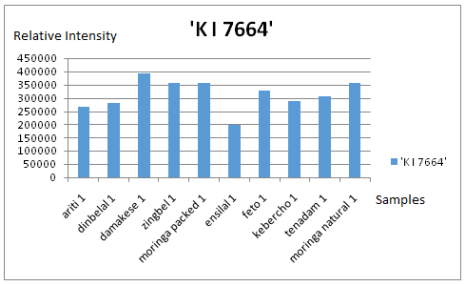


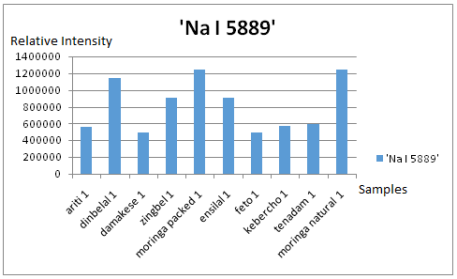












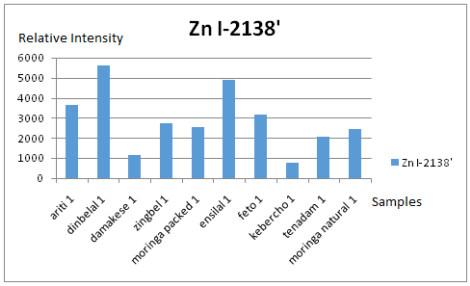


Figure 7.2: comparisons of mineral elements in terms of relative intensity of the emission lines

Comparatively ***Artemisia afra*** (Ariti) is rich in calcium and magnesium; ***Ocimum lamifolium*** (Demakese) is rich in magnesium and potassium; ***Zingiber officinale*** (Zingibil) is rich in magnesium, manganese, phosphorous and potassium; ***Moringa stenopetala*** (Shiferaw) is rich in calcium, iron, magnesium, potassium and sodium; ***Echinops kebericho*** (Kebercho) is rich in potassium; ***Foeniculum vulgare*** (Ensilal) is rich in iron, magnesium and zinc; ***Lepidium sativum*** *(*Fieto*) is rich in magnesium, copper and potassium;* ***Coriandrum sativum*** (Dimbelal) is rich in copper, magnesium, phosphorous, sodium and zinc; ***Ruta chalepensis*** (Tenadam) is rich in calcium and magnesium,

Many researches have done on elemental analysis of herbal medicines as indicated in the literature parts of this thesis. For the case of comparison we have displayed the result of the research done by Abuye (Abuye et al., 2003), on compositional study of *Moringa stenopetala* leaves on table 7.2 and our results on table 7.3. The study was performed by flame photometer and flame atomic absorption spectroscopy.

Table 7.1: Mineral contents of raw leaves of *Moringa stenopetala*

Minerals	Mg/100g
Na	403.5 ± 21
K	453.0 ± 11
P	65.6 ± 13
Ca	792.8 ± 92
Fe	3.08 ± 0.8
Zn	0.53 ± 0.8

Table 7.2: Amount of minerals in leaves of Moringa stenopetala in terms of integrated relative intensity

Minerals	Amount in terms of integrated relative intensity
Na	1.946854381 ± 0.0005
K	1.97501673 ± 0.0004
P	0.560437659 ± 0.0002
Ca	2.05238402± 0.0003
Fe	0.100298074 ± 0.0002
Zn	0.00401033 ± 0.0001

From these, in terms of the amount of mineral elements it can be seen that the result is in agreement with what we have found in our researches.

7.3 Correlation of mineral elements in herbal medicines

Calcium is essential for the formation of strong bones and teeth and for the maintenance of healthy gums. It increases the rate of bone growth and prevents against bone loss associated with osteoporosis. Calcium is important in the maintenance of a regular heart beat and transmission of nerve impulses. It helps lower cholesterol levels and helps prevent against cardiovascular disease and certain forms of cancer including colorectal cancer. Calcium is important for normal blood clotting processes that aid in the early stages of wound healing. In addition, calcium also wards off the accumulation of an excess of acid or alkali in the blood. It is involved in the activation of several enzymes including lipase, which breaks down fats for utilization by the body (Balch, J.F. and P.A, 1997 Barney, 1998; Dunne, 1990).

Magnesium is an essential nutrient required for many biologic functions in the body, including more than 300 enzyme reactions. It also functions in the activation of amino acids, the syntheses of DNA, and is involved in neurotransmission and immune function. Numerous studies show that a magnesium deficiency may be an underlying cause of cardiovascular disease, hypertension, asthma, chronic fatigue and pain syndromes, depression, insomnia, irritable bowel syndrome, and many pulmonary disorders. Supplementing the diet with magnesium may also prevent depression, dizziness, muscle weakness, twitching, and premenstrual syndrome (PMS). It promotes the absorption and assimilation of other minerals including calcium, phosphorous, sodium, and potassium while enabling the utilization of vitamin B complex and vitamins C and E (Fischer P., Kubena K. 2006; Balch, J.F. and P.A, 1997; Dunne, 1990).

Sodium is essential for maintaining blood pH and proper water balance. Together with potassium, it helps regulate the distribution of fluids on either side of the cell walls. Sodium and potassium are also intricately involved in muscle contraction and expansion as well as nerve stimulation. During intense exercise or extreme heat, sodium activates the thirst response. It keeps the other blood minerals soluble so that a buildup of other minerals will not accumulate in the blood stream. Sodium also acts with chlorine to improve blood and lymph health and aids in eliminating carbon dioxide from the body (Balch, J.F. and P.A, 1997).

Iron is involved the production of hemoglobin and myoglobin. Hemoglobin carries oxygen from the lungs to the body. Iron is essential for many enzymes and is important for growth, proper cognitive function. Iron is vital in energy production and in maintaining an optimal immune system (Hunt, 2006).

Potassium is a key for a healthy nervous system, regular heart rhythm, and proper muscle function. It is necessary for chemical reactions within the cells and helps in maintaining normal

blood pressure and in generating electrochemical impulses. In persons with hypertension, potassium can dramatically lower both systolic and diastolic pressure. It functions in cell metabolism, enzyme reactions and the synthesis of muscle protein from amino acids in the blood. It works with phosphorous to send oxygen to the brain and functions with calcium in regulating neuromuscular activity. Potassium will also stimulate the kidneys to eliminate poisonous wastes. It is also necessary for healthy skin (Balch, J.F. and P.A, 1997; Dunne, 1990).

Manganese is a component of several enzymes and, therefore, acts as a catalyst in the synthesis of cholesterol and fatty acids, and plays a role in protein, fat, and carbohydrate production. It activates a number of other enzymes including formation of cartilage in the bone and skin. Manganese is important for the production of milk, formation of urea, or part of the urine. It also maintains sex-hormone production, nourishes the nerves and brain, and is essential for the formation of thyroxin, an important component of the thyroid gland (Dunne, 1990; Montvale, 2001).

Phosphorous is needed for proper bone and tooth formation, cell growth and contraction of the heart muscle; it assists in the assimilation of vitamins and the conversion of food into energy. It also works with calcium to maintain the calcium-phosphorous balance in the bones. Deficiency of phosphorous can cause lack of appetite and weight loss (Dunne, 1990 Nielsen and Dunn, 2006).

Zinc has a variety of functions in the body. It is a component of at least 25 enzymes involved in digestion and metabolism, including carbohydrate digestion, and phosphorous metabolism. Zinc is essential for general growth and proper development of the reproductive organs and prostate gland function. It also may help prevent acne and control the activity of oil glands. It aids in the synthesis of protein and collagen formation, promotes a healthy immune system, aids in wound

healing and allows for enhanced vision, taste and smell. Zinc is a component of insulin and many vital enzymes. It will fight and prevent against the formation of free radicals. Zinc also increases the absorption of vitamin A (Dunne, 1990; Balch, J.F. and P.A, 1997; Montvale, 2001; Cousins).

Copper aids in the formation of bone, hemoglobin, red blood cells. It aids in the conversion and transport of iron from the intestinal lumen into red blood cells. It works in balance with zinc and vitamin C to form elastin. It is involved in the healing process, energy production, hair and skin coloring, and taste sensitivity. It is involved in the development and maintenance of the cardiovascular system. It also helps maintain the myelin, which sheaths nerves and aids in the transmission signals from the brain to the body and vice versa (Balch, J.F. and P.A, 1997).

7.4 Clustering of the herbal medicines using PCA

Moringa leaves are known to have a high content of protein, minerals and vitamin, hence an ideal nutritional supplement, (Fletcher, 1998). Moringa leaves are also considered a rich source of minerals (Gupta et al, 1989). In this work we have tried to characterize our herbal medicine samples by using principal component analysis as shown in figure 7.3. From the figure it is clearly observed that *Foeniculum vulgare* (Ensilal), Ruta chalepensis (Tenadam), Coriandrum sativum (Dimbelal), Artemisia afra (Ariti) and Hagenin abyssinica (Fieto) have similar elemental composition as moringa because these samples are clustered together with moringa. This classification model obtained by PCA may be of great use for quality inspection of raw herbal material on a continuous basis as new one is produced.

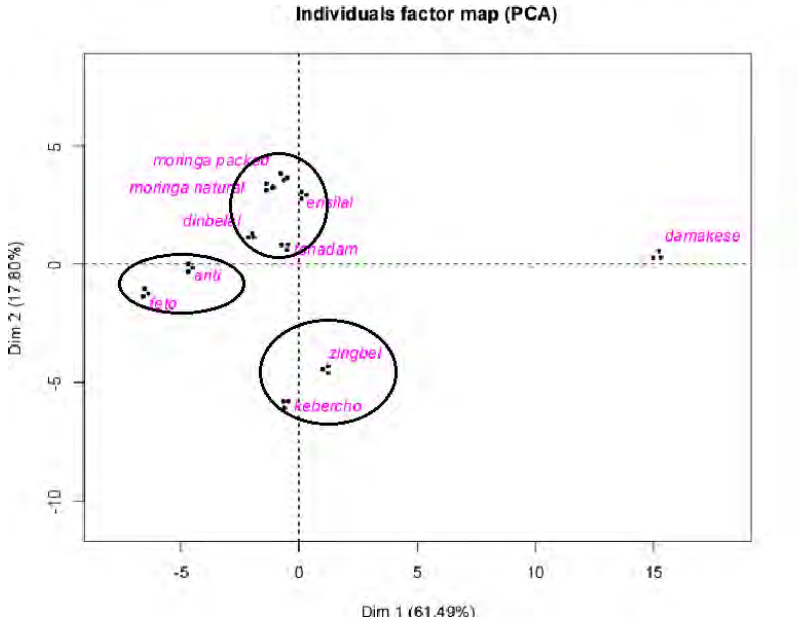


Figure 7.3: Clustering of the herbal medicines using PCA

7.5 LIBS used to monitor the change in elemental compositions of the mixed herbal medicines.

A well-defined and constant composition of the herbal medicines is most important prerequisites for the production of a mixture of herbal medicines. In this research we have demonstrated that LIBS can be used to monitor the change in elemental compositions of the mixture of herbal medicines.

We prepared samples by mixing two types of herbal medicines in equal amount. Sample11 is the mixture of *Ocimum la mifolium* (Demakese) and *Foeniculum v ulgare* (Ensilal). *Ocimum lamifolium* (Demakese) is relatively r ich i n p otassium and foeniculum *vulgare* (Ensilal) is relatively rich in zinc. Sample 12 is the mixture of *coriandrum sativum* (Dinbelal) and *zingiber officinale* (Zingibil). *Coriandrum s ativum* (Dinbelal) is relatively r ich i n c opper and *zingiber officinale* (Zingibil) is relatively rich in manganese. We analyzed the mixtures by using LIBS

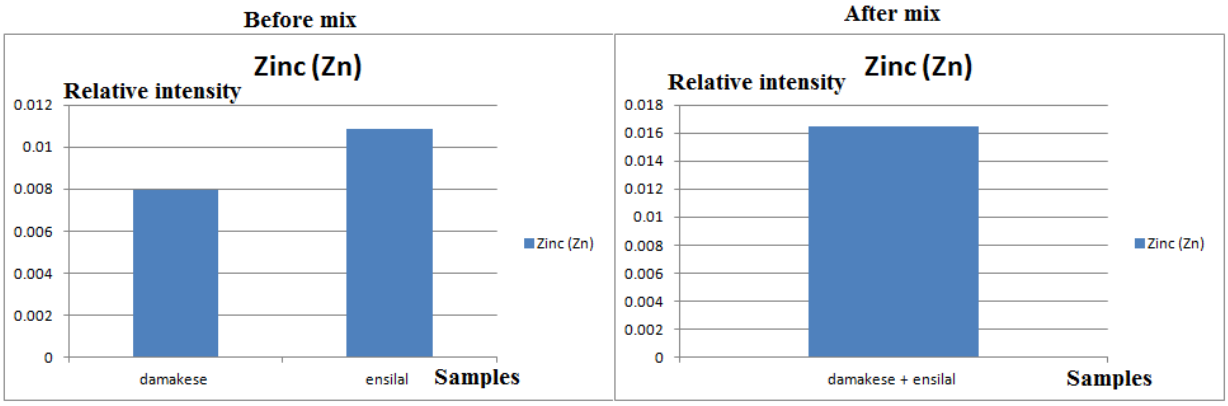
and we have displayed the results before and after mixing by using table 7.3, table 7.4 and histograms in figure 7.4 and figure 7.5

Table 7.3: amount of mineral elements in terms of the normalized relative intensity before mix

Sample	damakese	ensilal	dimbelal	zingbel
Potassium (K)	2.647387 ± 0.0023	0.447015 ± 0.0002		
Zinc (Zn)	0.007945 ± 0.0002	0.010856 ± 0.0001		
Copper (Cu)			0.057957 ± 0.0002	0.011775 ± 0.0001
Manganese (Mn)			0.166925 ± 0.0002	0.858159 ± 0.0002

Table 7.4: amount of mineral elements in terms of the normalized relative intensity after mix

Sample	damakese + ensilal	dimbelal + zingbel
Potassium (K)	3.07221± 0.0035	
Zinc (Zn)	0.016501± 0.0003	
Copper (Cu)		0.069732 ± 0.0002
Manganese (Mn)		1.003063 ± 0.0003



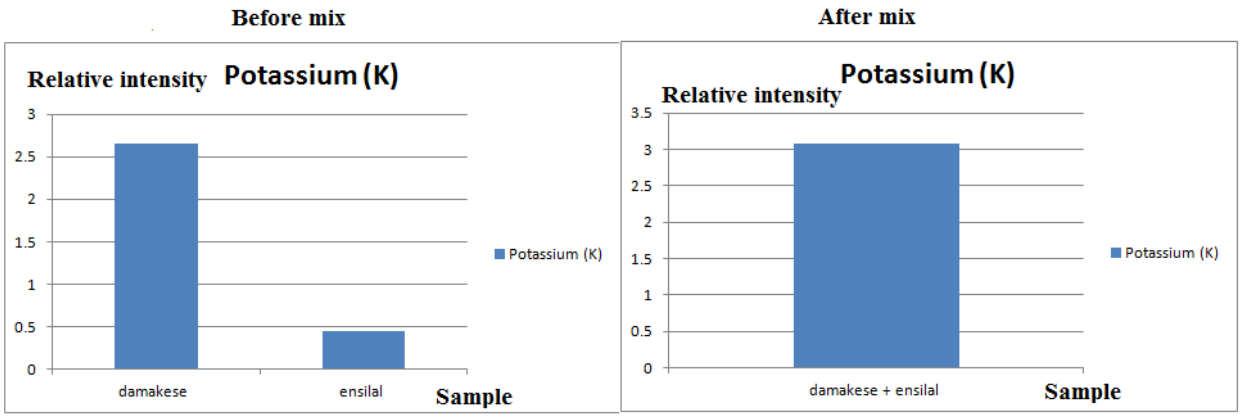
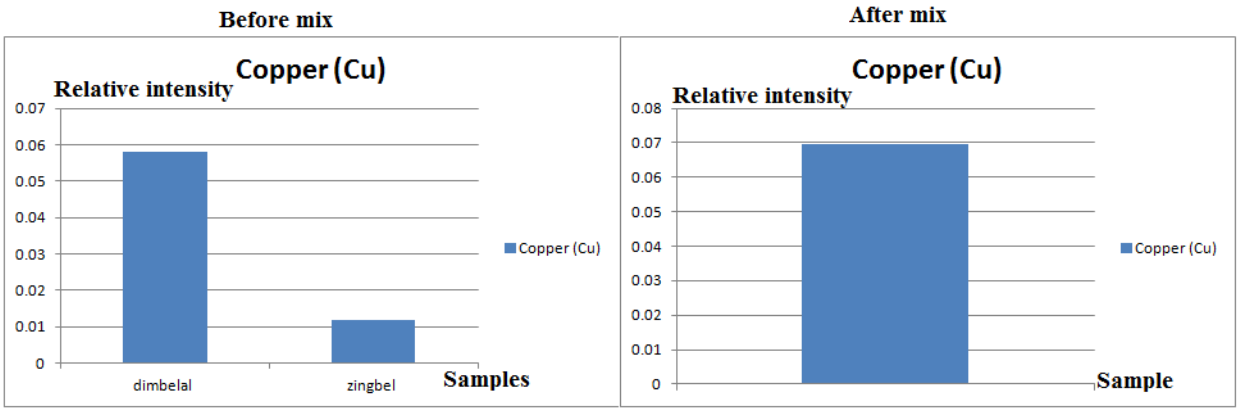


Figure 7.4: change of Zn (above) and K (below) of sample 11 in terms of relative intensity of the emission lines

As we observe from figure 7.4 for sample 11 the increase in zinc (Zn) using the relative intensity unit is 51.99% compared to the amount of zinc in Ensilal before the mixing and the increase in potassium (K) using the relative intensity unit is 16.05% compared to the amount of potassium in Damakase before mixing.



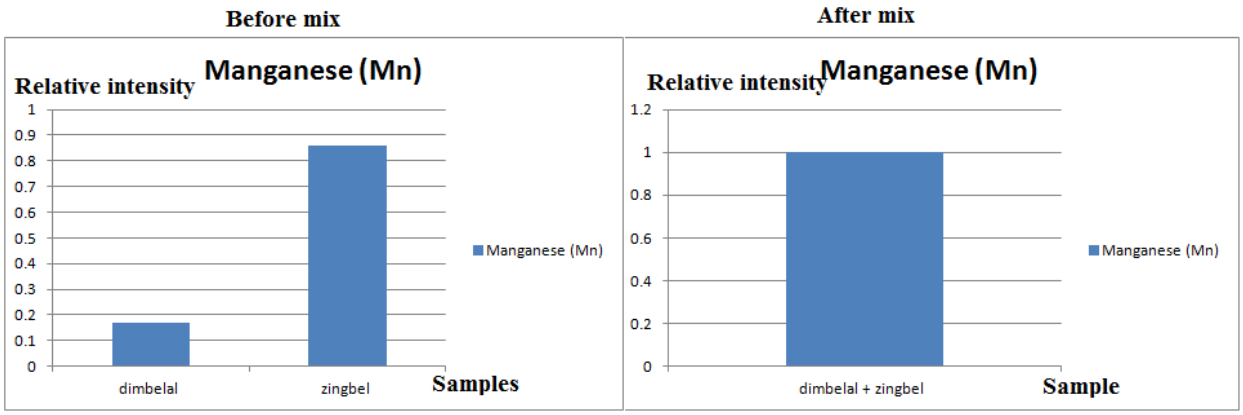


Figure 7.5: change of Mn and Cu of sample 12 in terms of relative intensity of the emission lines

Figure 7.5 shows result for sample 12, the increase in manganese (Mn) using the relative intensity unit is 16.88% compared to the amount of manganese in Zingbel before the mixing and the increase in copper (Cu) using the relative intensity unit is 20.31% compared to the amount of copper in dimbelal before mixing. This may show the capability of LIBS in monitoring the change in elemental composition of the mixture of herbal medicines. This can be of help in deciding the quantity of various active constituents and also supervising the dose of a particular formulation since dosage has been the main problem facing herbalists.

8

Conclusions

In our researches we have demonstrated the potential of LIBS in analysis and characterization of coffee utilizing multivariate chemometric method. Using the most relevant emission signals of LIBS, an attempt was made in order to discriminate the samples of coffee and their mixtures using Principal Component Analysis. We applied PCA for characterization of Ethiopian coffee, according to their geographical origin first by using forty nine atomic and ionic lines then by using all calcium and magnesium lines. We investigate several ranges and identify the use of these models on a specific range for a higher accuracy; accordingly we can able to characterize the coffee samples by using two lines of magnesium and two lines of calcium and finally we optimize our characterization by using only one line of calcium and one line of magnesium. By taking a sample at a time we have shown the capability of LIBS coupled with PCA for discrimination of coffee samples from their mixtures and this is useful for inspection of adulteration of coffee. In this research we have clearly shown that the Ca and Mg lines are the emission lines for discrimination of Ethiopian coffee samples.

In this research we have demonstrated the potential of LIBS in elemental analysis, characterization and monitoring the change in elemental composition of herbal medicines. We have pointed out that the medicinal plants studied are a source of biologically important elements, which may play part in the observed therapeutic properties of these plants. The classification model obtained by PCA may be of great use for quality inspection of raw herbal material on a continuous basis as new product is produced. We have also shown the capability of LIBS to monitor the change in elemental compositions of the mixtures of herbal medicines. This study can be of help in deciding the quantity of various active constituents and also supervising

the dose of a particular formulation since dosage has been a chief problem confronting traditional herbalists. The findings of this study can thus assist herbal medicine practitioners in their efforts to include these plants into a variety of formulations based on their mineral composition.

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