INSTITUTO POLITÉCNICO NACIONAL
CENTRO DE INVESTIGACIÓN EN CIENCIA APLICADA Y TECNOLOGÍA AVANZADA

# APLICACIÓN DE LA TÉCNICA FOTOACÚSTICA AL FORTALECIMIENTO DE AGRICULTURA LIMPIA

**TESIS**

Que para obtener el grado de

Doctor en Tecnología Avanzada

presenta:

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**DIRECTOR**
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**MÉXICO, 2011**

INSTITUTO POLITÉCNICO NACIONAL
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TECNOLOGÍA AVANZADA

# PHOTOACOUSTIC TECHNIQUE APPLIED TO THE STRENGTHENING OF CLEAN AGRICULTURE

Submitted in Partial Fulfillment of the

requirements for the

Ph.D. Degree in Advanced Technology

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**MEXICO, 2011**

SIP-14

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En la Ciudad de México, D.F. siendo las 11:00 horas del día 12 del mes de Septiembre del 2011 se reunieron los miembros de la Comisión Revisora de la Tesis, designada por el Colegio de Profesores de Estudios de Posgrado e Investigación de CICATA-Legaria para examinar la tesis titulada:

Aplicación de la técnica fotoacústica al fortalecimiento de agricultura limpia

Presentada por el alumno:

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Después de intercambiar opiniones, los miembros de la Comisión manifestaron ***APROBAR LA TESIS***, en virtud de que satisface los requisitos señalados por las disposiciones reglamentarias vigentes.

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# RESUMEN

Gran parte de los actuales cambios climáticos tienen origen no solamente en los productos de la combustión en vehículos y plantas industriales, sino también en el mal manejo de suelos, y en la contaminación que se hace del agua y del aire a través del uso inadecuado de pesticidas y de abonos químicos. Todo esto ha llevado a un gran desequilibrio en los diferentes ecosistemas. Para la solución del problema ambiental es de gran importancia el apoyo y la asistencia científica y tecnológica a nuevas formas de cultivo amigables con el medio ambiente y la optimización de procesos de postcosecha que den un valor agregado a los productos. Las técnicas fototérmicas (FT) han demostrado ser muy eficientes para la caracterización térmica y óptica de materiales, así como para el estudio de procesos fotobáricos, lo cual permite su utilización para estudiar materiales orgánicos. En este trabajo fue usada la técnica de espectroscopia fotoacústica (FA) en el rango visible e infrarrojo del espectro electromagnético para obtener información acerca del contenido de pigmentos y composición de café y arroz orgánico respectivamente, que sirven para identificarlas en un proceso de discriminación. Por otra parte, se obtuvieron parámetros termofísicos de esos mismos productos y de Guadua (un abundante ecomaterial) usando la técnica FA. Estos parámetros están estrechamente relacionados con la composición química y la estructura del material; y son útiles para la obtención de modelos para optimizar procesos de transformación y discriminación. Cuando la muestra presenta adicionalmente actividad fotoquímica, como es el caso de las hojas de las plantas, los cambios de presión debidos a la posible producción de gases contribuyen a la señal FA, fenómeno que fue usado para evaluar el efecto de la aplicación de biofertilizantes a plantas de maíz y de café a través del monitoreo de la razón de evolución de oxígeno en el proceso fotosintético. También fue evaluado el efecto bactericida de películas de dióxido de titanio sobre bacterias promotoras del crecimiento de plantas con el objetivo de identificar comportamientos típicos que permitan su identificación y caracterización.

# SUMMARY

The most of the weather changes taking place in the last decades have their origin not only in the combustion products of vehicles and industrial plants but also in the bad management of soils, and in the water and air pollution via inadequate use of pesticides and chemical fertilizers. This has led to non-equilibrated ecosystems. The assistance of science and technology to environmental friendly agriculture and optimization of post crop processes is very important for solving these environmental problems. The photothermal (PT) techniques allow the spectroscopic analysis of organic opaque samples and their thermal characterization, so that they give valuable information for industry and commercialization processes. Photoacoustic (PA) spectroscopy measurements and correlation analysis that allow proposing a discrimination criterion of powdered coffee and rice are reported in this work. Also, the thermophysical parameters of organic green coffee, organic rice and Guadua (an eco-material from Centro and South America) were measured with the PA technique in order to study the relation of these parameters with the cellular structure of the material. When the samples additionally present photochemical activity, as in leaves of plants, the pressure changes by periodical generation of gases are added to the PA signal. This effect was used in this work to evaluate the effect of biofertilizers on oxygen evolution rate in the photosynthesis process of coffee and maize plants *in situ* and *in vivo*. Using the same method, the bactericidal effect of titanium dioxide films on plants growth-promoting bacteria was evaluated for identifying typical behaviors that serve for the purpose of characterization.

# ACKNOWLEDGMENTS

I am grateful to Dr. Ernesto Marín, my major advisor professor, for his efficient supervision and guide, and especially by his kind and friendly personal attendance during the development of this work at CICATA-IPN.

The Members of the advisory committee, Dr. Antonio Calderón, Dr. José Guzman, Dr. Miguel Angel Aguilar Frutis, Dr. Alfredo Cruz, and Dr. José Antonio Irán Diaz Góngora, are also greatly acknowledged by carefully reading of the manuscript, and by useful comments and help for improving this work.

I also gratefully thank the fellowship of "Universidad del Quindío- UQ" and the scholarship of COLCIENCIAS, which was managed by the Academic and Professional Programs for the Americas (LASPAU). Especially, I acknowledge the Manager of Advising Services of LASPAU, Lisa Mallozzi Tapiero and the Placement Coordinator of LASPAU, Gaelle Brachet-Barro.

I am grateful to the members of my research group (GICADE-UQ) by technical support: F. Zárate, C. Mejía, D.M. Cortés, A.J. García, A.F. Bedoya, K. Villa, J. D. Duque, J.A. Ortega, C. García, J.S. Cuartas, L.F. Marín, J.A. Herrera and L. Y. Rivera ("Biointegrados"). The author acknowledges valuable assistance of Dr. H.S. Ariza-Calderón from Sciences Interdisciplinary Institute- UQ.

The moral support of the photothermal group at CICATA-Legaria is also acknowledged. Arlem Lara is especially thanks for her unconditional help.

The technical assistance of Leticia Cardona and Laura Y. Vega Haro with administrative processes, of Alfonso Martinez with the SEM measurements, of Rocío Sanchez and Gerardo Cruz with library services, all from CICATA-Legaria, and of L. Martinez-Aguilar from "Centro de Ciencias Genómicas", UNAM-Morelos, for technical support with the preparation of samples used in Chapter 4, is also greatly acknowledged.

I also thank the support of Dr. M Zapata, Dr. C Mora and of COFAA-IPN through the PIFI Program.

The partial financial supports of CONACyT through research grant 83289 and of SIP-IPN through grants 20090160 and 20100780 are also acknowledge.

I am thankful to Dr. Gabriel Peña of “Universidad Francisco de Paula Santander” for suggesting me the possibility of a doctoral stay at CICATA-Legaria under the advisement of Dr. E. Marín.

Posthumous acknowledgment is given to Dr. Jesús Caballero-Mellado from “Centro de Ciencias Genómicas”, UNAM-Morelos-Mexico, by his legacy to biofertilizers development and especially for his uninterested collaboration with one part of this work.

Finally, I thank to my wife, María Hersilia and my son David Fernando for their patience and company during this stage of my professional career.

*For Hersilia and David*

# CONTENTS

# INTRODUCTION

Agricultural activity highly contributes to the pollution of water and air especially since the so-called “green revolution” due to the increasing use of agrochemicals and agrotoxic products in large areas dedicated to important crops such as rice, maize, coffee, etc. These toxic products also affect the health of the agriculturalists and consumers [1]. Furthermore, the industrial production of synthetic fertilizers requires large quantity of fossil fuel, which increases the environmental negative impact [2]. On the other hand, the over-exploitation of forest resources affects the ecosystems and diminishes the amount of $O_2$ that is produced by the trees.

The organic agricultural techniques and the use of timber alternatives for eco-building are some strategies for minimizing the environmental damage and negative effects of conventional agro-forest practices in human health. However, the first option requires certification and enough efficient production for guarantying sustainability, while the second one requires full characterization for standardizing industrial processes in order to replace conventional timbers. Sustainability and lower cost of organic products and eco-building materials can be obtained using biotechnological developments such as “biofertilizers”[3], which are made with plants growth-promoting bacteria, and using fast growing forest resources such as *bamboo*. Unfortunately, the effect of these beneficial bacteria is still not approved by the most of producers. Thus it is necessary to evaluate the efficiency of biofertilizers directly on plants for understanding metabolism changes [4]. In this way, the manufacture process of these products can be optimized so that they can gain credibility as helpers for the adequate absorption of nitrogen from atmosphere and nutrients from organic or chemical fertilizers, avoiding leaching of fertilizers and use of pesticides. On the other hand, organic products are certificated through expensive protocols that finally give an over-price to the final product for an exclusive consumer, so that these foods are not accessible for the most of the people.

Some practices of post-harvest of organic as well as conventional crops cause pollution of potable water natural sources. The treatment of waste-water must be a requisite for responsible and sustainable agricultural production. However, the treatment methods are expensive and conditions of high efficiency are a subject of research since some years.

Advanced oxidation processes should be particularly used for environmental friendly production [5]. Specially, high efficiency photocatalytic oxidation methods with solar light for reducing costs of practical applications have been proposed, which must be still evaluated in an adequate way.

The photoacoustic (PA) technique has become especially important for characterizing materials of biological source because the physical processes involved in the PA signal generation can carry up information about light absorption, light-energy into heat conversion mechanisms, heat transport, pigmentation and chemical composition [6], among others. Furthermore, the possibility of sensing the contribution of photochemical activity opens a way for measuring "*in vivo*" and "*in situ*" the photosynthetic activity. Clearly, the production processes of commodity are strongly involved to the agricultural activity. Thus, the application of the PA technique has enormous potential for improving evaluation criteria of agricultural cultivation treatments, and for discriminating products of different origin with special characteristics. If the environmental impact of agricultural practices is considered, these action fields are pertinent and this potential is still more appreciated.

PA can be applied as a cheap and efficient technique to partially resolve the agro-physics problems that were commented before, which are related to plants physiology and characterization of foods. The relatively low cost of this technique suggest it as an appropriate tool for certification of organic products and evaluation of new materials for improving the plants growing and degrading contaminants in waste-water. With this non-destructive technique, thermal characterization, visible and infrared spectroscopy and monitoring of photochemical processes *in situ* can be done. PA thermal characterization does not use electrical contacts, PA spectroscopy eliminates light interference and diffusion problems and preparation of samples becomes simple without the use of any sample´s matrix, photochemical processes can be monitored without measuring of gas pressures and especial cases of photosynthetic processes can be studied *in vivo*.

In this thesis, four specific problems of cleaner agriculture were explored with the use of the PA technique. Firstly, the possibility of certification of organic products was explored through the particular case of coffee and rice. Secondly, the evaluation of the effect of growing promoting bacteria on plants, in particular of coffee and maize, were considered.

Thirdly, the thermal characterization of the *Guadua angustifolia*, called the “bamboo of the Americas”, since the use of ecological construction materials like it can be important for avoiding the exclusive use of classical tree woods. Finally, the photocatalytic activity of titanium dioxide on plants growing promoting bacteria, looking for a cheap method for characterizing these strains.

This thesis has been organized as follows. In chapter 1, a description of the theoretical fundamentals of the PA technique is done. Arguments for the application of this technique to thermal characterization, spectroscopy, and photochemical process measurements are described. The others five chapters describe the principal obtained results

In chapter 2, the application of the PA technique to organic coffee certification is discussed. Coffee consumption has become a subject of great interest because of the amplitude of the related market. Growing, process and consume have become a whole culture and there are several types of coffee classified according to geographical origin, variety, growing techniques, toasting degrees, quality, etc. Appropriate organoleptic characteristics can be chosen by selecting one of these or a combination of them according to personal tastes. Although several studies about how conventional agricultural, industrial and consumer’s practices can affect people’s health, coffee continues being one of the most popular beverages in the world. It is clear, for example, that toxic chemical products applied to crops are often inoculated in the bean. Thus there are levels that cannot be exceeded without involving a serious risk to consumer’s health. Nowadays old agricultural techniques called “organics” are applied with the help of biotechnology avoiding the use of toxics products. This has obvious positive consequences for consumer health and environment. Besides that, the adulteration and degradation of coffee samples is another aspect of interest. Thus, monitoring the quality of coffee is an important area of research, although an in-depth look into the scientific literature on this subject shows that the majority of the studies are limited to the investigation by means of spectroscopic and chemical analysis methods of the chemical composition of coffee and how properties such as its aroma and flavor depend on it. This chapter will be mainly focused to spectroscopy and thermal characterization of grains and powders of roasted coffee. PA spectroscopy monitoring of the photosynthetic activity of coffee plants is a useful tool for studying the effect of bacterial inoculants application, which allows better nutrients absorption and control of pests with minimal environmental problems from leaching of N fertilizers. On the other hand the knowledge of thermal parameters is particularly important for the improvement of the industrial roasting of coffee as well as for monitoring the degradation of the final product when, for example, it is exposed to cyclic periods of heating that can affect its quality. We will also show how coffee plants photosynthetic activity, coffee beans thermal parameters, the presence of pigments and content of chemical components in ground and roasted coffee can have a different behavior depending on growth conditions.

In chapter 3, important differences between organic and conventional samples of rice are argued for possibly use in a certification process. Rice (*Oryza sativa*) is a staple food for more than half of the world population. This cereal contributes a considerable amount of calories to the consumer. Agrotoxics and synthetic fertilizers in agricultural practices also have generated environmental damage and the pollution of fruits, which puts consumer health at risk. On the other hand, as commented in chapter 2 for coffee, organic agricultural production requires certification, which is frequently a complicated and expensive process of inspection. For this reason, it is pertinent to look for a scientific discrimination criterion that allows for the certification procedure to be done in an efficient and economical way. In this chapter, a PA spectroscopy study for husk and white grain rice samples of *Combeima* variety is shown. The samples were taken from crops of rice that were cultivated with organic and conventional techniques. Thermophysical parameters of the same kind of samples were measured with the same technique, but in a modulation frequency-resolved configuration. Finally, rice grain cell morphology was studied by optical microscopy.

In chapter 4, the evaluation of the effect of two growing promoting bacteria on maize plants was considered. Conventional agricultural practices cause damages to soil principally by eutrophication processes, contributing in great manner to the current weather changing. Particularly in the maize (*Zea mays*) producer regions, the growth of these plants in large soils extensions affects their sustainability. Some microorganisms living in roots convert nutritionally important elements through biological processes for making these available to the plant. The use of this mutual interaction has a great potential to improve the nutrient absorption, which avoids leaching problems. However, the physiological effect of microbial fertilizers application has to be studied for understanding the mechanisms and interactions among these microbes and the plants. In this chapter, the time resolved PA technique was used to measure the photobaric process in plants from seeds which were inoculated with two beneficial bacteria, namely *Azospirillum brasilense* and *Burkholderia unamae*, for comparing their photosynthetic activity. The results showed different values of oxygen diffusion coefficient, oxygen evolution rate, and leaves thermal diffusivity in a group of plants grown under the action of these “biofertilizers” respecting to a control group.

In chapter 5, looking for other agricultural product of high economic and environmental potential, we consider *Guadua agustifolia* Kunth (*Guadua a.* for short). This is a giant graminea native of Central and South America. This plant captures a lot of carbon dioxide and protects hydrographic watersheds. For this reason, the growing of *Guadua a*. is considered environmentally favorable. Furthermore, the use of this forest resource as a structural and decorative element for building has been promoting recently due to its special physical characteristics. However, nowadays much of the production chain is inefficiently oriented by empirical and traditional knowledge. In particular, the drying process exerts influence on cracks that disqualifies the material for some artistic and industrial applications. Thermal characterization allows making models for drying according to particular characteristics of the material. Knowledge of thermal properties is also important for applications in which heat transfer can play an important role, such as in buildings. In this chapter, thermal diffusivity was measured as a function of the moisture content obtained during the drying process in samples of *Guadua a*. taking from the bottom, middle and top culm regions of the plants. Measurements were performed using the PA technique. Results showed that thermal diffusivity increases with the moisture content but its value becomes the same along the bamboo. This behavior is highly correlated with the morpho-anatomical characteristics of the plant, which were determined through scanning electron microscopy. For the interpretation of experimental results, thermal effusivity measurements have been also performed using the same PA technique aided with a model based in the well known analogy between electrical and thermal phenomena.

In chapter 6, the photocatalytic activity of titanium dioxide on growing promoting bacteria was studied using the PA technique looking for a cheap method of characterization. This activity has been evaluated in waste water. When Titanium dioxide in contact with a polluted water sample is irradiated with ultraviolet light, electron-hole pairs can be generated, which can react with oxygen and water producing free radicals that can degrade the pollutants, changing them into harmless compounds for the environment. The ultraviolet component of the solar radiation is around 7%. Therefore, it is convenient to modify the $TiO_2$ films crystalline structure for obtaining photocatalytical processes with visible light. In this chapter we report results about the growth of $TiO_2$ thin films by the Sol-gel technique considering the incorporation of $AgNO_3$ in the initial solution containing the precursor to dope it with Ag. The shift in the forbidden energy bandwidth value to the visible region of the optical absorption spectrum was evidenced by PA Spectroscopy. The photocatalytic activity was tested on a solution of methylene blue using also the PA technique and its bactericidal effect on plants growth beneficial bacteria was studied.

## Hypothesis

The adequate use of the three modes of operation of the photoacoustic technique, namely resolved in wavelength, in modulation frequency and in measurement time, could be useful tools for the study of environmental problems related to "clean agriculture".

## Objectives of the thesis

### *Main objective:*

To prove the potential of the photoacoustic technique for contributing to the strengthening of “clean agriculture”.

***Specific objectives:***

1- To find information, using the PA technique, about characteristics of organic coffee for defining possible certification criteria.

2- To use the PA technique for monitoring the quality of coffee.

3- To monitor the photosynthetic activity of coffee plants using the PA technique as a way to study the effect of bacterial inoculants application.

4- To find information about characteristics of organic rice using the PA technique that can help to define possible criteria for certification.

5- To use the time resolved PA technique for measuring the photobaric process in maize plants from seeds inoculated with two beneficial bacteria: *Azospirillum brasilense* and *Burkholderia unamae.*

6- To measure the thermal diffusivity as a function of the moisture content obtained during the drying process in samples of *Guadua angustifolia* taking from the bottom, middle and top culm regions of the plants.

7- To test the photocatalytic activity of titanium dioxide films on a solution of methylene blue using the PA technique.

8- To study the bactericidal effect of titanium dioxide films on beneficial bacteria of plants using the PA technique.

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# CHAPTER 1

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## FUNDAMENTALS OF PHOTOTHERMAL TECHNIQUES

## 1.1 Introduction

More than 100 years ago Alexander Graham Bell (1847-1922) [1] discovered that an acoustic signal can be generated in a sample enclosed in an air filled cell by the absorption of intensity modulated light. This is the Photoacoustic Effect [2]. The PA effect was discovered by Bell while working, together with Charles Sumner Tainter (a known manufacturer of optical instruments), in the photophone (Fig. 1.1), according to Bell an instrument more revolutionary than the telephone that he had patented some years before [3], and with which he intended to transmit voice to great distances using sunlight as a carrier of information.

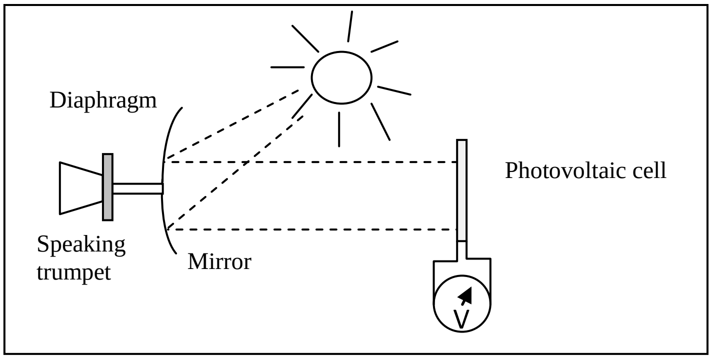

**Fig. 1.1.** In the photophone the light is reflected onto the photovoltaic cell. The electric current produced by this cell varies with the vibration of the mirror, which is caused by oscillations from speaking trumpet.

Bell reflected a beam of sunlight onto a selenium cell incorporated into a telephone circuit (during a trip to England in 1878 he had studied the properties of selenium, including the changes in its electrical resistance when it absorbs light). The beam was reflected with the help of a mirror placed in the diaphragm of a kind of speaker so that it can vibrate activated by voice. The electrical resistance of selenium was then modulated by the absorbed light, reproducing the transmitted voice towards a telephone receiver (note that Bell, with these works, anticipated a century to what is today the transmission of information over long distances through the atmosphere, and introduced the idea of optical communication that caused later a scientific revolution with the development of optical fibers). The photophone was introduced at a meeting of the American Association for the Advancement of Science, held in August of 1880 in the city of Boston, becoming then the first patent on wireless telephone communication [4]. Immersed in his experiments with the photophone, and placing the Selenium in the form of a diaphragm on a listening tube, Bell discovered that this material (and other solids) emits sound when it is illuminated by modulated light, obtained by passing a light beam through a rotating disc with holes (a mechanical chopper). Bell even discovered, using a device called by him spectrophone (Fig. 1.2), that the intensity of the emitted sound depends on the wavelength of the incident light, so that the effect should be attributed to a process of optical absorption.

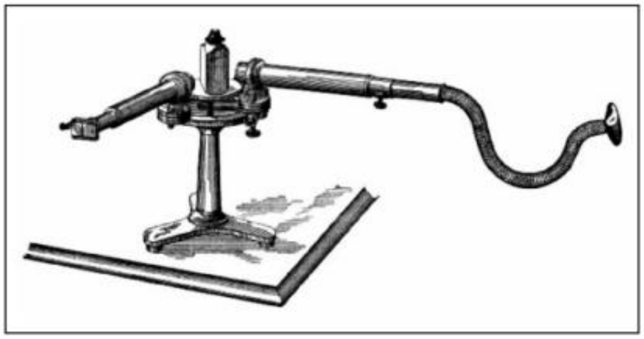

**Fig. 1.2**. Spectrophone designed by Bell.

Although the PA effect in solids gained the interest of some eminent scientists of these times, such as Röntgen (he discovered that the effect could be also produced in gases), Tyndall and Lord Rayleigh, it remained as a scientific curiosity for almost half a century until that, thanks largely to the development of the microphone that would replace the listening tube in the experimental setup of Bell, the advent of intense light sources (e.g. the lasers) and the development of sensitive systems of detection (e.g. synchronous amplifiers) and data-processing (computers), the first practical applications began to gestate [5, 6]. Nowadays, the PA and other so-called photothermal (PT) techniques [7], which have been inspired in it, have found several applications [8]. Among them thermal characterization of materials and monitoring of dynamic processes by “hearing” with the help of photoacoustics is an exciting area of research.

## 1.2 The photothermal techniques

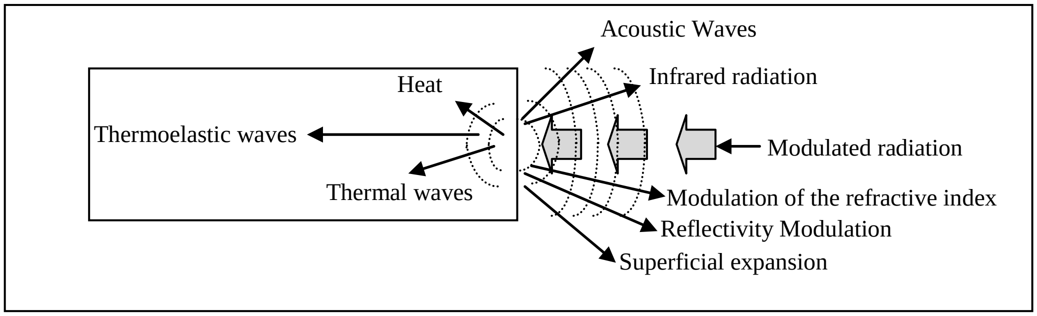

**Fig. 1.3** Scheme of PT effect. Detection of temperature variations in the sample or its surrounding is the basis of the PT techniques.

The PT techniques are a group of experimental methods based in a common principle of perturbing the thermodynamic state of a sample by periodical, transient or step heating [9] (generated often by optical energy absorption), and measuring the generated temperature changes that are called by some authors thermal waves (Fig. 1.3). These depend on the optical properties of the sample (because they vary with the light wavelength this dependence allows spectroscopic applications), on those determining the light into heat conversion processes (e.g. by the photochemical effect) and on thermal properties such as thermal diffusivity, $\alpha$, and effusivity, $\varepsilon$. Thermal waves can be detected directly or indirectly. In the first case this can be performed by measuring changes in temperature dependent parameters, such as the sample optical reflectance, refraction index, etc. In the PA technique, for example, the thermal waves are measured indirectly through acoustic waves that are induced by them in the sample or in the media surrounding it. On the other hand, the temperature changes can be measured directly using special kinds of thermometers, such as infrared detectors (in the PT radiometry method) or pyroelectric sensors attached to the sample in the method known as the photopyroelectric (PPE) technique [10]. Many other variants have been proposed during the last 30 years.

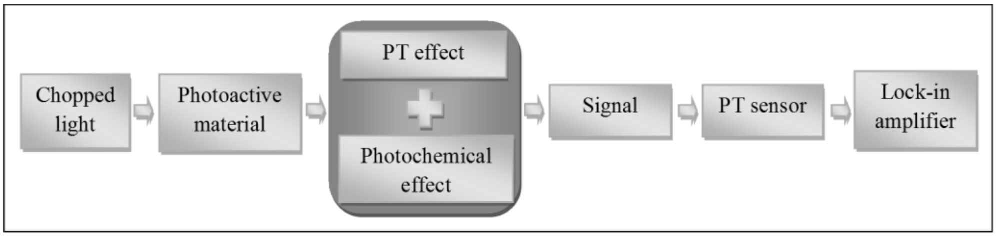

**Fig. 1.4.** A block diagram of a typical experimental set-up for experiments performed in the modulation frequency domain.

The Fig. 1.4 shows schematically a basic experimental set-up. Using mechanical chopping the intensities of the radiation sources employed (high intensity polychromatic light lamps and a monochromator for spectroscopic applications, and continuous laser sources for non-spectroscopic measurements) fluctuate periodically in the form of square or sine waves, resulting in 50% duty cycles. The resulting electrical oscillatory signals generated by the PT sensors (see next sections) will be analyzed in the frequency domain using synchronous lock-in amplifiers. The whole apparatus is computer controlled for easy data acquisition and processing.

Because this thesis will be deal mainly with the PA technique, a brief description of it will be given bellow. The discussion will be limited to the particular case of interest here in which thermal waves are generated by the absorption of periodical intensity modulated light at a given frequency, $f$.

## 1.3. The photoacoustic technique

There are several models for the description of the PA signal generation process. The most accepted of them, the RG model, also known as the heat diffusion model, has been proposed by Rosencwaig and Gersho [11] in 1976. It is well described in detail elsewhere [12, 13]. Different cases (Fig. 1.5) are analyzed in this model by comparing three characteristic lengths, namely the sample´s thickness, $L$, the light penetration length, $\mu_\beta=1/\beta$ (i.e. the inverse of the optical absorption coefficient, $\beta$) and the thermal diffusion length, defined as $\mu=(\alpha/\pi f)$. Here $\alpha$ is the thermal diffusivity. This parameter is related to other relevant thermal properties of a material, namely the thermal conductivity, $k$, the thermal effusivity, $\varepsilon$ and the specific (volume) heat capacity, $C=\rho c$, where $\rho$ is the density and c the specific heat. The four parameters are related to one another by means of two mathematical relationships (see Table 1.1). Therefore, it is sufficient to measure two of them to known all. While thermal conductivity and specific heat can be measured using stationary and static methods respectively, the thermal diffusivity and the effusivity can be determined using dynamical techniques, such as the PA ones [14]. Due to this reason, and because thermal conductivity and specific heat are well known parameters for the majority of people, only the physical meaning of the thermal effusivity and diffusivity is outlined in Table 1.1. Because $\beta$ depends on the light photons wavelength, by changing this parameter $\mu_\beta$ can be varied as well for a given sample, whereas $\mu$ can be controlled by adjusting the modulation frequency. This renders noticeable experimental possibilities. A sample is defined as thermally thin when the condition $L<<\mu$ is fulfilled and thermally thick when $L>>\mu$. On the other hand the sample can be optically opaque ($\mu\beta<<L$) or transparent ($\mu\beta<<L$) (see Fig. 1.4).

**Table 1.1.** Thermal properties characterizing non-stationary heat transfer by conduction.

| Parameter | Description | Relationship |
|---|---|---|
| Thermal diffusivity | It defines the spatio-temporal shape of the temperature field in a sample exposed to non-stationary heating. It is related to the rate of heat propagation in the sample. | $\alpha = \frac{k}{C} = \frac{k}{\rho c}$ |
| Thermal effusivity | It determines the thermal wave amplitude at the the surface of a sample heated by a periodical modulated heat source. | $\varepsilon = \sqrt{k\rho c} = \frac{k}{\sqrt{\alpha}}$ |
| Thermal diffusion length | It is the distance at which a thermal wave amplitude decreases $e$ times respect to its value at the surface of the sample heated by a periodical modulated heat source. | $\mu = \sqrt{\frac{2\alpha}{\omega}} = \sqrt{\frac{\alpha}{\pi f}}$ |

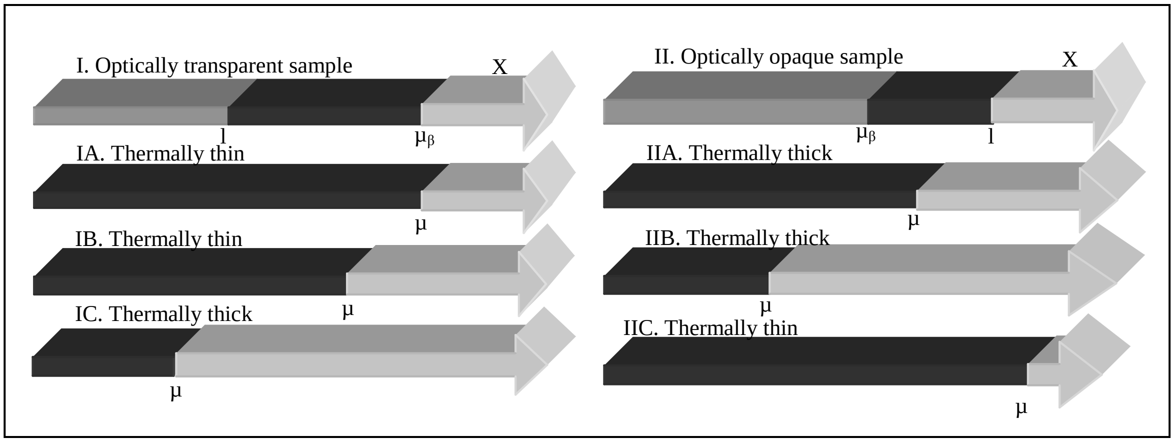

**Fig. 1.5.** Particular cases of interest in the PA technique with modulated light excitation and front detection configuration.

Consider that the sample is heated on the same surface at which the PA signal is detected. For optically semi-transparent samples (Case I of Fig. 1.5) the signal depends on the product $\beta L$ when the sample is thermally thin and on $\beta\mu$ if it is thermally thick. These products determine the distance from which useful information can be obtained and suggest the possibility of spectroscopic applications. In the second case, for example, only light absorbed within the thermal diffusion length contributes to the signal. For thermally thin samples (cases IA and B) only the particular situation IIB, for which the sample becomes thermally thick with the thermal diffusion length smaller than the optical penetration depth, allows spectroscopic measurements.

Respecting thermal properties determination the case of optically opaque samples (II) is the relevant ones. As a consequence of the physical meaning of thermal diffusivity and effusivity given in Table 1.1, the measurement of the former parameter is common using a heat transmission configuration in which the PA signal is detected at the rear non-illuminated surface of the sample, while thermal effusivity is better achievable by sensing the PA signal at the same surface of the sample where it is generated.

In this method the main part of the experimental setup is a gas (often air) tight cell (the PA cell), in which both a sample and a microphone are enclosed. It is illustrated in the Fig. 1.6. The heat generated by the absorption of light will diffuse through the sample reaching a very thin layer (about one thermal diffusion length thick) of the gas in contact with it, which will be also periodically heated so that it can expands and contracts following the light modulation frequency and acts as a piston on the rest of the gas inducing acoustic waves. These can be detected by the microphone. This is the principal mechanism of generation of the PA signal and is well described by the RG model, allowing applications of the technique in the field of spectroscopy and for thermal characterization, as mentioned above. Contrary to other optical methods the PA signal is insensitive to diffuse light, so that not only homogeneous solid samples can be characterized: Powders can be analyzed as well. This fact opens several possibilities for the characterization of powders of roasted and soluble coffee, a form in which this product is often commercialized.

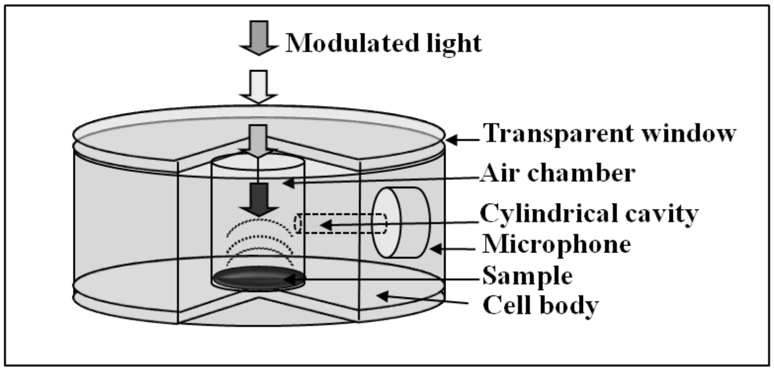

**Fig. 1.6.** Schema of a conventional PA cell with frontal detection.

While the configuration show in the Fig. 1.6 is the most conventional ones, being often applied for spectroscopy and for measurement of samples in the form of powders, for thermal diffusivity measurements of thin solid samples (such as leafs) many authors resort to the use of a heat transmission configuration. A cheap variant to achieve it is using the so-called open PA cell (OPC) method. The PA cell is formed by a commercial electret microphone (Fig. 1.7) which uses its own chamber as the acoustic cell. The sample closes the (PA) chamber with the help of vacuum grease around the microphone hole to get it hermetically sealed. The microphone is composed on a metalized electret diaphragm and a metal plate separated by an air gap and connected through a resistor. The modulated light is focused onto the sample. Pressure oscillations in the air chamber deflect the membrane, generating a voltage across the resistor, which is supplied to a FET pre-amplifier already built in the microphone capsule. The signal is then sent to a Lock-In amplifier synchronized at the modulation frequency, where it is measured in amplitude and phase. From the obtained curves of these parameters as a function of $f$ the thermal diffusivity can be determined straightforwardly by fitting to a theoretical formula resulting from the RG model [14], which for an optically opaque and thermally thick sample predicts that the PA signal amplitude, $A$, can be expressed as [15]:

$$\log\,(A\times f) \approx -L(\pi f/\alpha)^{1/2} = \left(\frac{f}{f_c}\right)^{1/2} \qquad (1.1)$$

where $L$ is the leaf thickness and $f_c$=($\alpha$ /$\pi$ $L^2$) is the so called cutoff frequency. Thus from the slope of a semi-logarithmic plot of the product $A\times f$ as a function of $f^{\,1/2}$ the thermal diffusivity can be straightforwardly calculated.

More experimental details about this measurement configuration are given elsewhere [16].

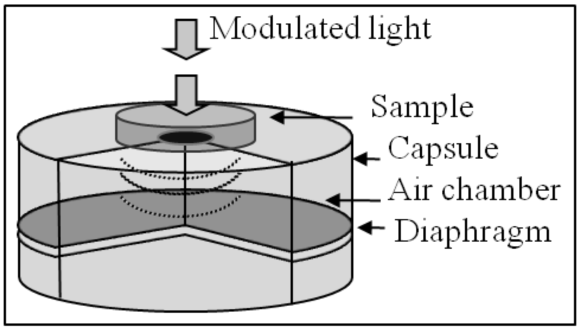

**Fig. 1.7.** Open PA cell for heat transmission detection mode.

Among the heat diffusion there are other factors that can influence the PA signal, among them gas evolution from the sample due to different causes. This contribution is of particular importance for the study of photosynthetic activity of green plants, such as coffee. Plants incorporate $CO_2$ and $H_2O$ to produce carbohydrates and $O_2$ in a process induced by sunlight. When a leaf is incorporated into the PA air chamber and irradiated with intensity modulated light the $O_2$ evolves in a periodically way generating pressure waves that are superimposed to the thermally generated acoustic waves contributing to the total PA signal. A review on early works on how plants photosynthesis can be studied by photoacoustics has been published by Charland and Leblanc [17] and by Barja [18], the later focused in applications of the OPC configuration. Biological samples are very complex systems. In a plant leaf, for example, following light absorption by the pigments and energy transfer processes, three major processes can happen, namely fluorescence, heating and photochemistry, the later being responsible for the $O_2$ emission. These processes can be studied by photoacoustics. Because they are competitive to one another an increase in a quantum yield of one of them should produce a decrease in a quantum yield of the others. For example, it is possible to separate the $O_2$, called the photobaric (PB), signal from the thermal ones. Consider that the leaf sample is illuminated with both, a modulated monochromatic light beam (with a wavelength at which the leaf can absorbs light energy) and a continuous white light beam (it is convenient the use of a light source with a sun like blackbody spectrum). Using the later the modulated photochemistry can be damped, since all modulated light is transformed into heat. Subtracting the saturated PA signal from the total signal measured in absence of continuous light, the part of the absorbed energy stored into chemical intermediates can be evaluated. The photoacoustic methodology has been extensively used in the field of photosynthesis in studies on the effect of environmental stresses on plant physiology, on pigments presence in organisms and light energy transfer between pigments, among others (see for example [19] and references herein).

The resultant PA signal, S, is generated by pressure changes in the cell due to both the PT (that includes the photochemical-loss contribution by energy storage due to carbon fixation [20]) and the PB contribution (S=PT+PB). We will call S´ the PA signal obtained after the saturating effect. It will correspond only to the PT effect, so that we will write for it S´=PT. Although the addition of a non-modulated strong white light saturates the photosynthesis process, it generates a constant value in the oxygen evolution. Thus, for each sample the signal amplitude was measured, and the OER was estimated through the decrease percentage in the signal amplitudes (namely $A$ and $A'$ for the amplitudes of S and S´ respectively) when light was turned on, i.e.:

$$\mathrm{OER} = ( A - A' )/A = 1 - ( A'/A ) \tag{1.2}$$

The value of OER is affected by non-photosynthetic factors, such as the leaf internal anatomy, which affects the diffusion of oxygen through the leaf tissues [21]. In order to consider these effects we followed the methodology described by Poulet *et al.* [22]. These authors showed that for a modulation frequency range where an optically opaque sample becomes thermally thick (the sample's thickness, $L$, is much greater than the so-called thermal diffusion length) it is also possible to write

$$R = \frac{A - A'}{A'} = R_0 \exp\left[ -\pi^{1/2} \left( \frac{1}{D_0^{1/2}} - \frac{1}{\alpha^{1/2}} \right) l \, f^{1/2} \right] \tag{1.3}$$

where $R_0$ is a constant dependent on the detection system, $l$ is the average diffusion path length of heat and oxygen from the chloroplast to the boundary of the cell and $D_0$ is the mass diffusion coefficient of oxygen in the aqueous medium of the cell. From measurements of $R$ as a function of the modulation frequency the oxygen evolution rate can be determined straightforwardly if the leaf's thermal diffusivity is known.

## 1.3 References

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# CHAPTER 2

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## CHARACTERISTICS OF ORGANIC COFFEE OBTAINED VIA PHOTOACOUSTIC TECHNIQUE

## 2.1. Introduction

Several techniques have been used for characterizing coffee such as ultraviolet-visible (UV-Vis), infrared (IR) spectrophotometry and gases chromatography [1, 2, 3, 4]. Chemical composition, volatile compounds, adulterants, variety and toxic traces identification have been studied in this way. The reach of tools and data bases that can serve to identify coffee non-detected characteristics by taste-test is the principal interest of this investigation field. Other topics related to transformation process have been published as well [5]. Moisture content in the bean has been identified as the principal parameter for roasting level, while the grain size influences the percolation in the infusion preparation [6]. Both characterize in great manner the smell and flavor of coffee beverage.

Coffee is one of the most-consumed beverages in the world, which are prepared from roasted seeds, also called beans, of the plant with the same name. The health effects of coffee have been extensively studied to determine how coffee drinking can affects humans and also to establish its beneficiary effects [7, 8]. Properties of coffee beverages (such as flavor and aroma, but also chemical changes that can have important effects on health) can depend in a great extent on the characteristics of the whole production process, from plant cultivation to roasting, grounding and cooking to create a drink. Therefore it is very important to develop characterization methods for monitoring the effects of these processes on the properties of coffee plants, beans and final beverages. There are several conventional techniques available for that, the most of them based in spectroscopic chemical methods. But, as in chapter 1 was commented, there is another form of spectroscopy known as photoacoustic spectroscopy (PAS). One advantage of PAS is that it is nondestructive to the sample, which does not have to be dissolved in some solvent or embedded in a solid-state matrix, as in conventional methods [9]. They can be measured as they are. Among many applications these techniques can be used to study biological samples such as coffee. This chapter will be devoted to describe some of the possibilities in this field.

## 2.2. Organic and conventional coffee

Global economy has come to enrich the offer of coffee for the consumer. Today, it is possible to select a coffee in powder or beverage shape by accounting to its geographical origin, variety, friendly environmental management and fair trade character, or a mixture of them. For this reason a certification process is necessary in the market [10]. Specially, the classification according to the growing technique has an increasing interest because toxic substances must be avoided and because the protection of soils, rivers water and biodiversity are impetuous. The high cost of agrochemicals and the environmental conscious of small farmers motivate cultivation practices that include growing under shadow trees, compost use and pest natural repellents. The products obtained with this technique are called organics.

Organic products have enormous beneficial consequences for environment and health of consumer. Principally, the soil care avoids erosion and the non-use of pesticides and monoculture systems protect the biodiversity and potable water sources [11].

Coffee is one of the largest traded tropical agricultural products, involving a commercialization of around 6 million of tons worldwide. Only the organic coffee market is around 3 percent of the total ones, in spite of that it has a premium in the price [12]. Conventional coffee is growing as monoculture using synthetic fertilizers and pesticides that finally end in the rivers by leaching of residues causing water pollution and eutrophication [13, 14]. Monoculture intensive agricultural practices break ecosystem equilibrium; so biodiversity is diminished and consequently pest natural control is lost. Microfauna and macrofauna in the soil is severely affected with exposition to high temperatures and ultraviolet light from the sun, so that the quality of the soil becomes pour because it loses permeability [15]. Due to these reasons, conventional agricultural techniques are considered unsustainable over the long-term.

**Fig. 2.1.** Coffee cultivated using organics techniques. Crops and soils are protected from the sun rays by higher plants. Small plants are kept in a polycultive system that cares the biodiversity.

In Fig. 2.1 a picture about organic coffee crop is shown. Organic agricultural techniques are seen as an alternative to remediate damages caused by conventional techniques. However, it has been proved that these techniques applied to coffee are not economic sustainable for smallholders producers [16]. The principal argument is the expensive certification process of the organic products. Sometimes a double certification is necessary according to the rules of the importer country [17]. On the other hand, productivity of coffee can be lower without the use of synthetic fertilizers, while negative effects in the soil are the same than in the conventional crops. In the last twenty years, biotechnology has developed products using bacterial inoculants that dispose adequate quantity of nutrients to the plant avoiding leaching of residuals of compost or synthetic fertilizers [18]. So, it is possible to reconsider sustainability of organic agricultural products, in special of coffee.

The present chapter synthesizes some efforts made to solve this problematic of organic coffee production. First, microscopy and PA techniques are used for characterizing organic coffee looking for discrimination criteria in certification. Second, morphology and photosynthesis of coffee are studied for evaluating bacterial inoculants. On the other hand thermal properties determination can be useful for the characterization of coffee beans.

## 2.3. Thermal characterization of organic coffee and conventional coffee

Simulations of commodity transformation use thermal diffusivity as an important property of heat transport for modeling process of foods [19]. The simulation must be done for optimization of final product quality avoiding risks in the dried, roasted, cool, or froze of large quantities of raw material used in industry.

The specific heat measures the change in the internal energy per unit of temperature change of a sample; thus, if the density of a solid increases (or decreases) the solid can store less (or more) energy, and specific heat decreases (increases). As a result the product of both magnitudes remains almost constant. Thus, any variation in the value of the thermal conductivity will be reflected in the thermal diffusivity. While the former parameter can be determined using stationary methods, requiring a strictly knowledge of the amount of heat flowing through a material, what is in general difficult due to the presence of non-controllable heat losses, there are several dynamic methods that have been proposed for the direct measurement of $\alpha$, among them the PA method.

Thermal diffusivity is related to heat propagation quickness in a material and its value is very sensible to temperature and to structural and compositional changes in materials [20, 21], playing a very important role in non-stationary heat transfer problems and in materials science. Thus its determination, as that of other thermophysical parameters, can be used to identify special characteristics of coffee [22]. In the particular case of a coffee bean, the roasting process conditions such as time and temperature, which define some characteristics of the beverage [23], can also depend strongly on thermal diffusivity, as well as the characteristics of the beverage itself. Therefore, coffee bean thermal diffusivity measurements can be of importance for different applications.

### 2.3.1 Bean characterization

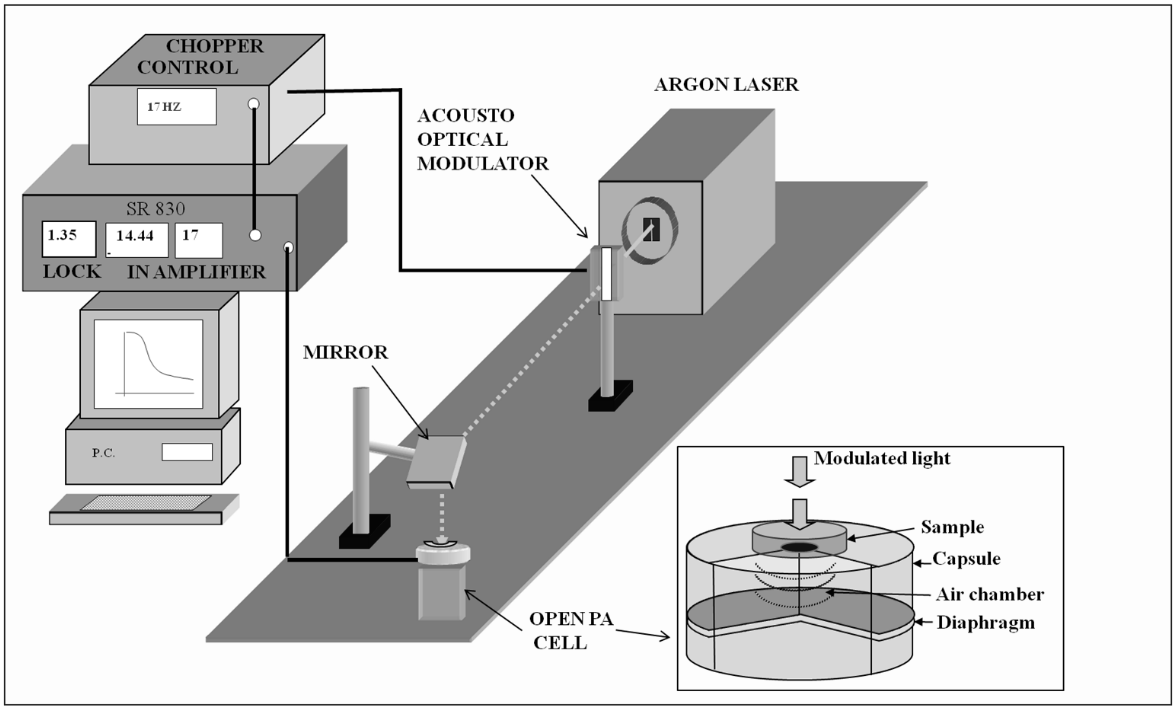

**Fig. 2.2.** PA system for diffusivity measurements. In the inset a representation of open PA cell section. Coffee sample is directly putted on microphone capsule.

The PA technique has been used for measuring thermal diffusivity in beans of conventional and organic coffee using the analysis described elsewhere [24]. The frequency resolved PA system configuration is described in Fig. 2.2. The PA signal amplitude is measured as a function of chopped light frequency using an open PA cell, in which the sample is hermetically adhered to the capsule of an electret microphone as described in section 1.3. In this case, light from an Argon laser (100mW, 514 nm, Modu-laser) impinges onto the sample after it was modulated by an optical-acoustic modulator (AA opto-electronic). It is absorbed by the sample surface and periodical heating is produced. The heat transported through the sample generates periodical heating of the gas enclosed in the microphone front chamber and therefore a sound signal. Thermal diffusivity was estimated as a fit parameter from the amplitude $A$ versus $f$ according to the Rosencwaig-Gersho (RG) model [25, 29].

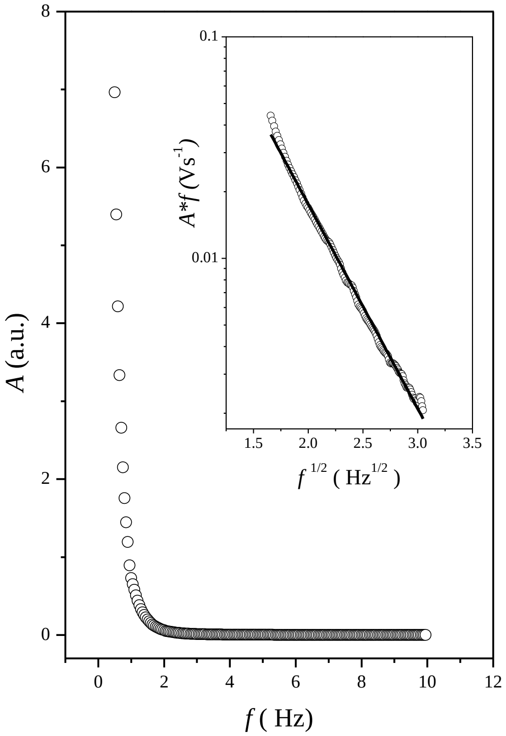

**Fig. 2.3.** PA signal amplitude as a function of the modulation frequency for a sample of organic coffee. Experimental points were fitted using the RG model (solid line in the inset).

Fig. 2.3 shows a typical curve obtained for an organic coffee sample. Measurements were done for fifteen beans of both organic and conventional samples and the obtained values of the thermal diffusivity were averaged. The solid line is the best least-square fit to Eq. 1.1. For organic and conventional coffee the corresponding mean values were estimated as $(10.8\pm0.2)\times10^{-8}$ $m^2/s$ and $(13.8\pm0.2)\times10^{-8}$ $m^2/s$ respectively [26]. This result indicates a remarkable difference that can be related to chemical composition and cell structure. Irregular cells size, in some cases non cell-wall, non-uniform cellular area and inhomogeneous cell interspaces are characteristics of conventional coffee, which were observed using optical microscopy. Cellular interspaces and cellular area had an average value for organic samples of $3.9\pm0.1\mu m$ and $25.2\pm0.2\mu m^2$ respectively, which were uniformly distributed through the bean. Furthermore, it was qualitatively evidenced from the microphotographs of coffee cellular structure shown in figures 2.4 and 2.5 that the lipids and amyloplasts content is higher in conventional that in organic samples. Histological stain was used to identify these characteristics through optical microscopy [27].

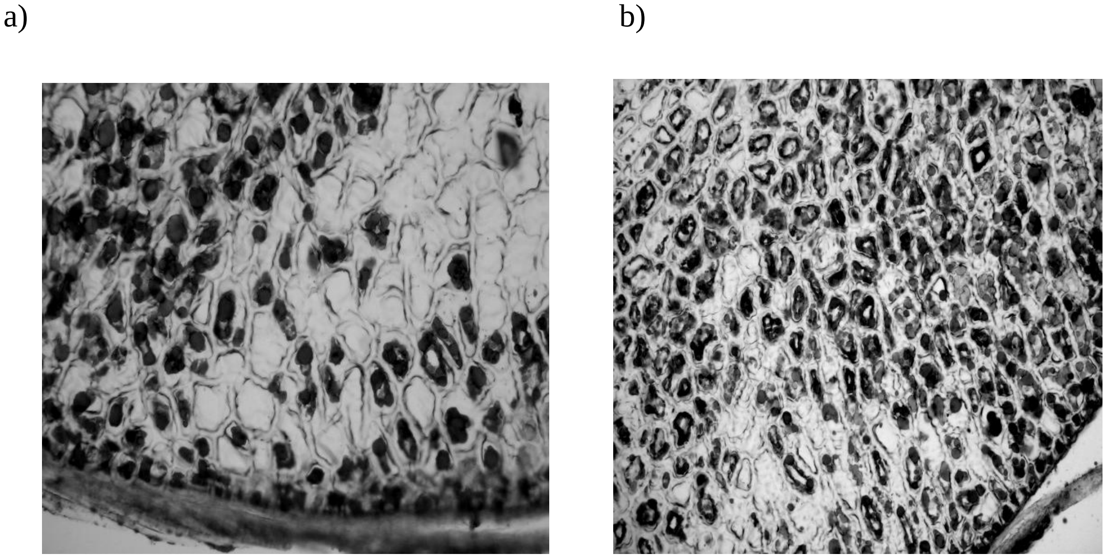

**Fig. 2.4.** Micrographs of coffee bean section stained with safranin. Lipids content in a) organic coffee and b) conventional coffee. The gray zones correspond to tissue where Sudan dye reacts with lipids.

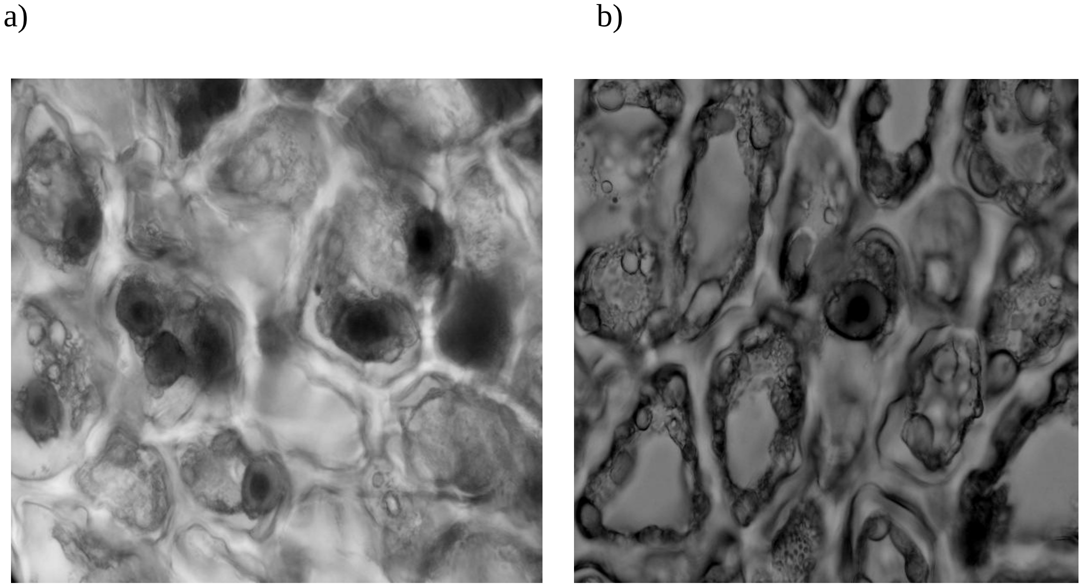

**Fig. 2.5.** Micrographs of coffee bean section stained with lugol. Amyloplast content in a) organic coffee and b) conventional coffee. The bubble shapes enclosing dark points correspond to amyloplasts reacting with Lugol dye.

### 2.4. Spectroscopic analysis of coffee

Spectrophotometry has been extensively used for authentication of coffee because the optical absorption spectrum of a given material can be considered as its fingerprint [28, 29, 30]. However, as mentioned before this method has some troubles such as light scattering and the necessity of incorporating the sample in a matrix, so that sample preparation can be complicated. For organic coffee discrimination and comparison between samples the use of high resolution techniques is necessary. Roasted and ground coffee have a high optical absorption coefficient in the visible range of the electromagnetic spectrum, as demonstrated by their intense color. This property is convenient to achieve a PA signal with high signal to noise ratio so that PA spectroscopy (PAS) can be used to resolve pigments that can be related to nutritive special characteristics of the sample [31].

On the other hand, using the same argument, PAS in the infrared range can resolve bands related to functional groups of the sample [32]. Thus, this method can be used for chemical composition studies that can supply useful information for the consumer. In this section, the use of visible range and mid-infrared (MIR) PAS for pigment content and chemical composition analysis of ground and roasted coffee will be described.

#### 2.4.1. Pigments in ground and roasted coffee: comparison between organic and conventional coffee

In the PAS system used for measurements in the visible range of the electromagnetic spectrum (Fig. 2.6), the chopped light from a 1000 W Xenon arc lamp (Oriel) is guided to a monochromator (TRIAX series 190). Light with the selected wavelength coming from the monochromator is then focused onto the sample, which is enclosed in a PA cell. The signal from an electret microphone coupled to the cell is fed to a lock-in amplifier (SR830) synchronized with the modulation frequency. The basic principle behind the generation of the PA signal is the same that has been described in chapter 1. According to the RG theory the PA spectrum is directly proportional to the sample’s absorption coefficient [33] and, as mentioned before, interference and light scattering are avoided with this technique, being this an important advantage respecting spectrophotometric methods [34]. To account for the spectral characteristics of the light source the measured PA signal must be normalized with the signal from a black body absorber, in our case a sample of carbon black.

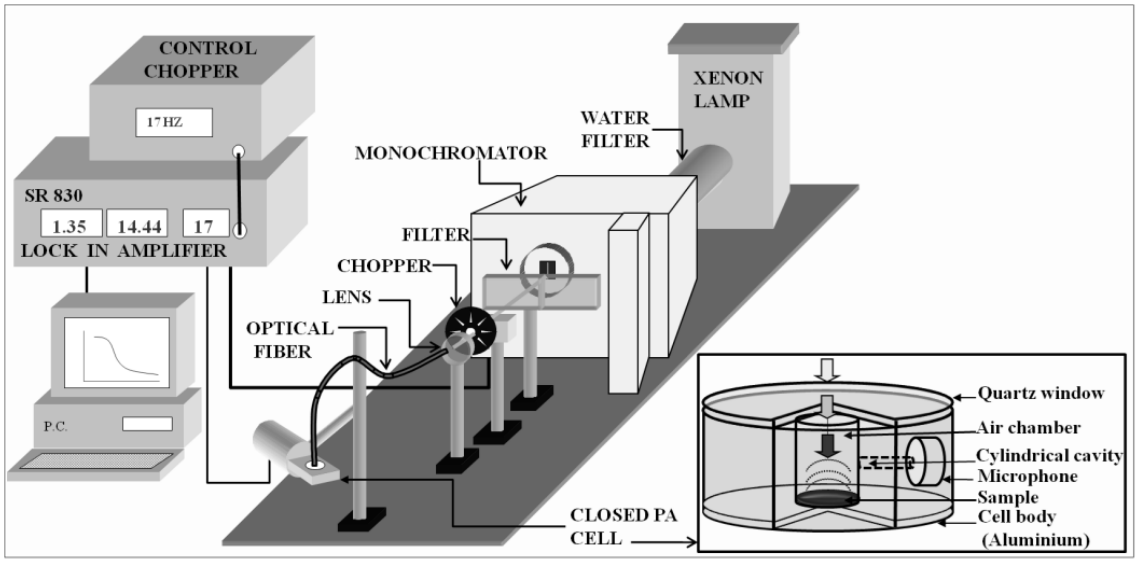

**Fig. 2.6.** PA spectrometer system. In this case the experimental arrangement is configured with constant modulation frequency and changing wavelength using a monochromator. In the inset a sectional view of a closed PA cell. For FTIR PAS a similar closed PA cell is putted in a spectrophotometer, but in this case a KBr window is used instead of a quartz window for eliminating infrared absorption.

| Dry coffee bean | Hulling | Roasted | Ground |
|---|---|---|---|
|  |  |  |  |

**Fig. 2.7.** Milling, roasting and ground process of coffee beans. After drying, with the milling of parchment skin (endocarp) the dried mucilage is removed.

Fruits from *Coffea arabica* were collected from plants cultivated on 1600 meters over sea level. They were depulped, demucilaged and dried until 12% of moisture in the bean was reached. The roasting process was done in a furnace by conduction between 200 and 210 ±1 $^0$C until moisture loss of 20%. Grain sizes were estimated between 250 and 315 µm after a ground process using sieves.

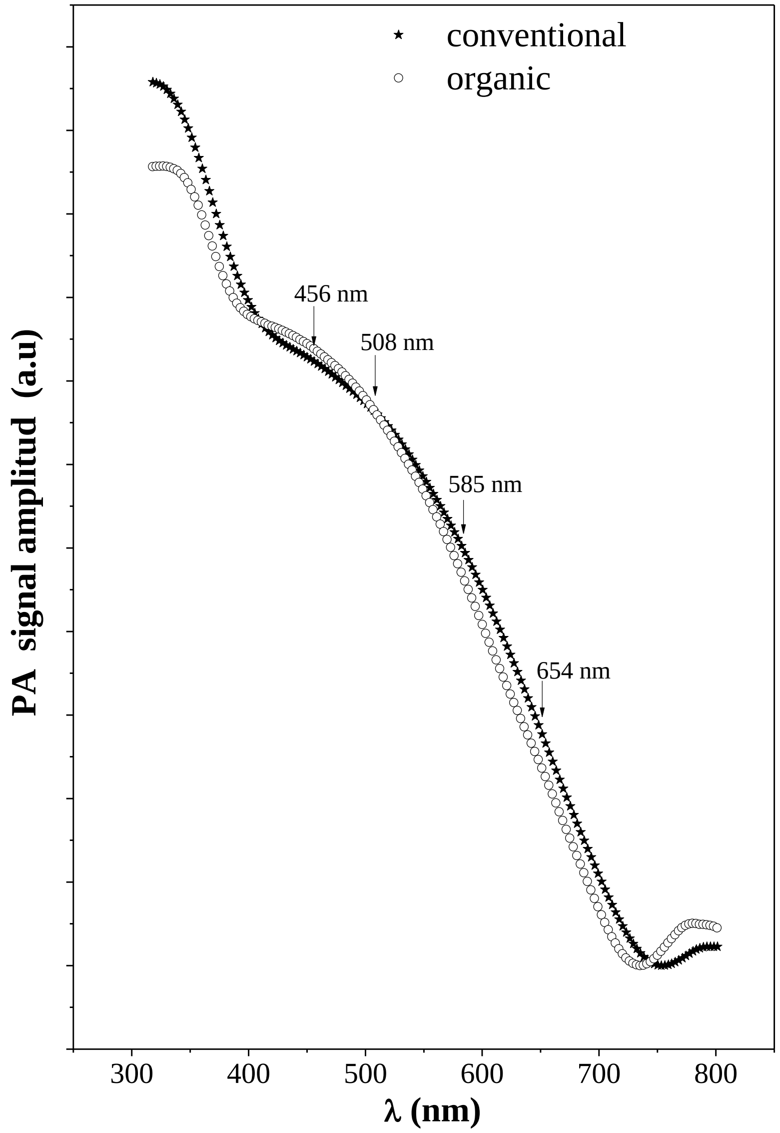

**Fig. 2.8.** Comparison between PA spectra of roasted and ground coffee prepared with beans of different cultivation technique: organic and conventional.

The Fig. 2.7 shows photographs of the results of the milling, roasting and ground process of the coffee beans. PA absorption spectra of roasted and ground samples for organic and conventional coffee are shown in Fig. 2.8. A simple look to the figure does not shows appreciable differences in the shapes of the curves, which can be defined by subtracting their first derivatives (Fig. 2.9) as shown in the Fig. 2.10. In this way a sample from excellent quality organic beans was compared with those prepared with conventional coffee beans. A panel of tasters certificated the quality of the investigated samples.

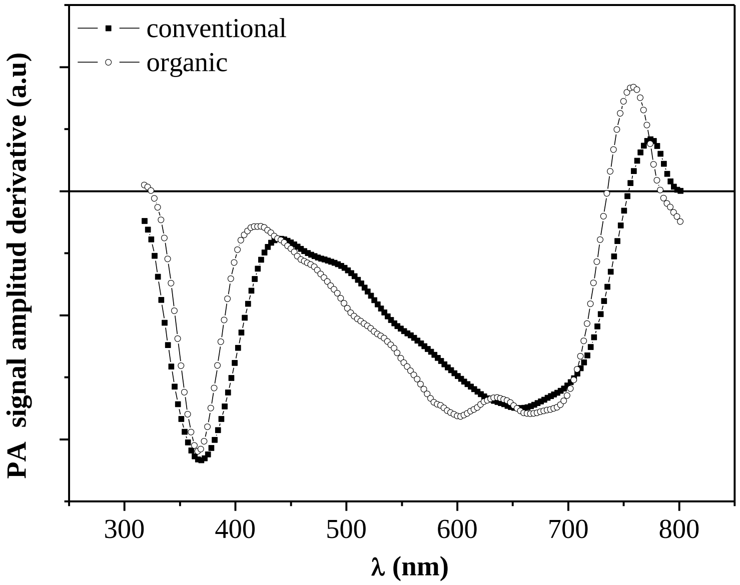

**Fig. 2.9.** First derivatives of spectra of roasted and ground coffee of Fig. 2.8.

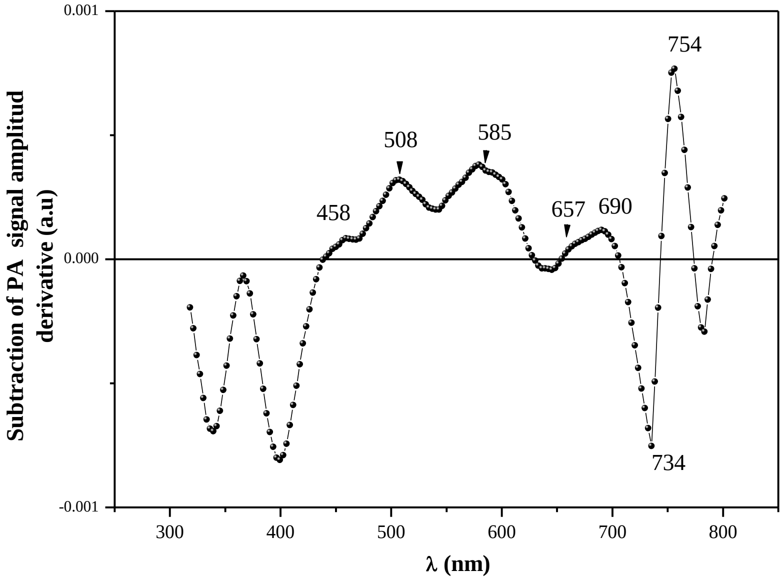

**Fig. 2.10**. Subtraction between the first-derivatives of the spectra described in figure 2.8.

A variance analysis (ANOVA) [35] was also used for comparing these spectra. The values of the F distribution as a function of the light wavelength (Fig. 2.11) indicate the intervals in which there are significant differences between the spectra, which are in agreement with the results obtained from the subtraction of the spectra. The observed shoulder at 456 nm is related to chlorophyll b and yellow carotenoids beta-carotene, zeaxanthin, and cryptoxanthin that determine the absorption; Absorption in the interval 505-590 nm corresponds to the red pigments capsanthin and capsorubin, while absorption at 654 nm is related to chlorophyll a. Specially carotenoids are known by its antioxidant nutritive function [36].

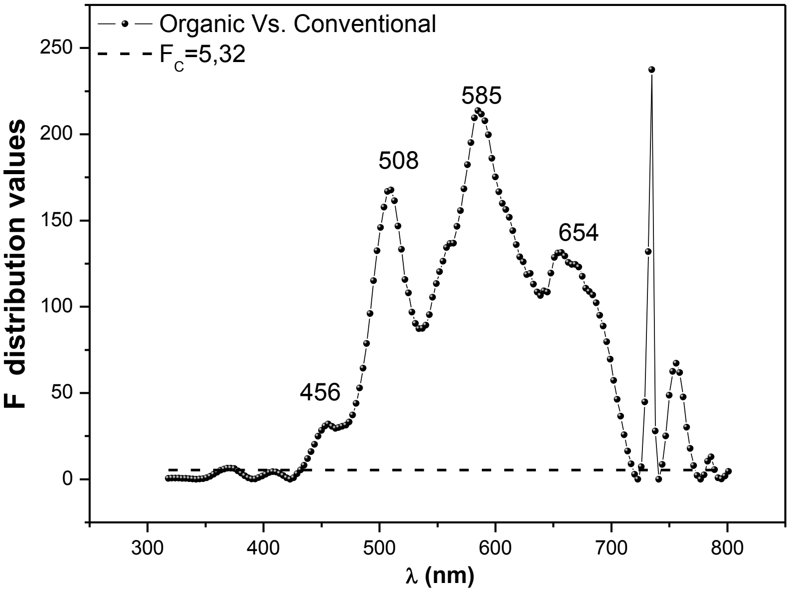

**Fig. 2.11.** F distribution values as a function of wavelength obtained from an ANOVA study of roasted and ground coffee spectra.

### 2.4.2 Quality identification

Since ancient times, coffee is one of the most consumed beverages worldwide. Flavor and smell are often evaluated in the determination of coffee quality. Organoleptics characteristics are related to the presence of bean defects or extraneous matter and to roasted process. The most important types of defects are represented by black, sour or brown and immature beans [37]. In this work, beans perforated by Broca worm, as well as black and brown beans were taken as samples in a PAS experiment.

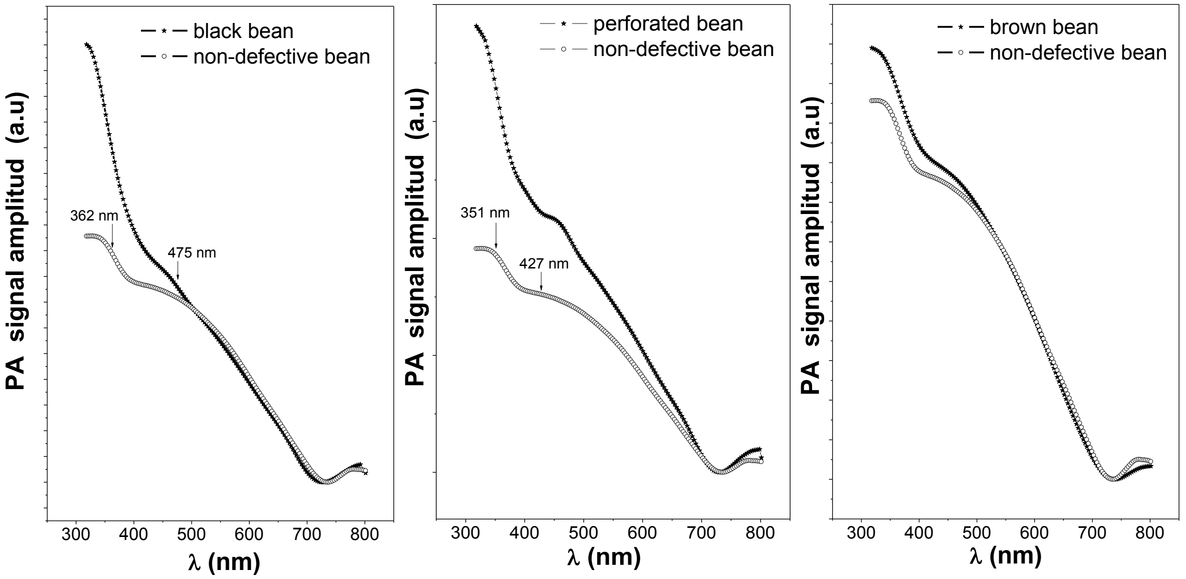

**Fig. 2.12.** Comparison between PA spectra of roasted and ground coffee prepared with beans of different quality: black bean, perforated bean, brown bean and non-defective bean.

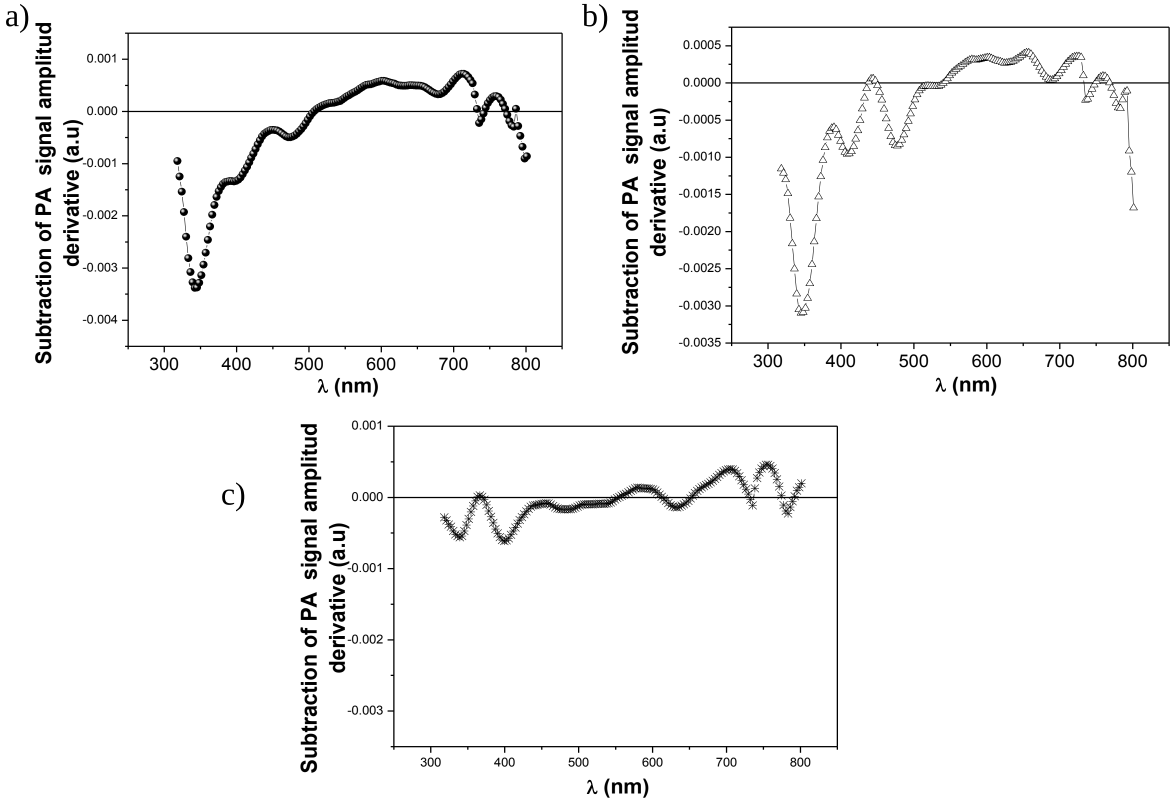

**Fig. 2.13.** Subtraction between the first-derivatives of the spectra showed in figure 2.12. Subtraction between: a) black bean and non-defective bean spectra; b) perforated bean and non-defective bean spectra and c) brown bean and non-defective bean spectra.

Fig. 2.12 shows average spectra corresponding to 10 measurements of each sample of roasted and ground coffee prepared with these beans and Fig. 2.13 and Fig. 2.14 evidence differences in the spectral curves shapes when each spectrum is compared with that corresponding to a sample prepared with excellent quality beans. Some wavelengths are related to absorption centers that have different behavior according the beans characteristics. At 362 and 475 nm there are important differences for samples prepared with black beans; at 351 and 427 there are difference for perforated beans; while brown coffee does not present significant differences respecting to non-defecting beans. So, after roasting and ground process it is possible to establish the quality of coffee beans through this pigmentation analysis.

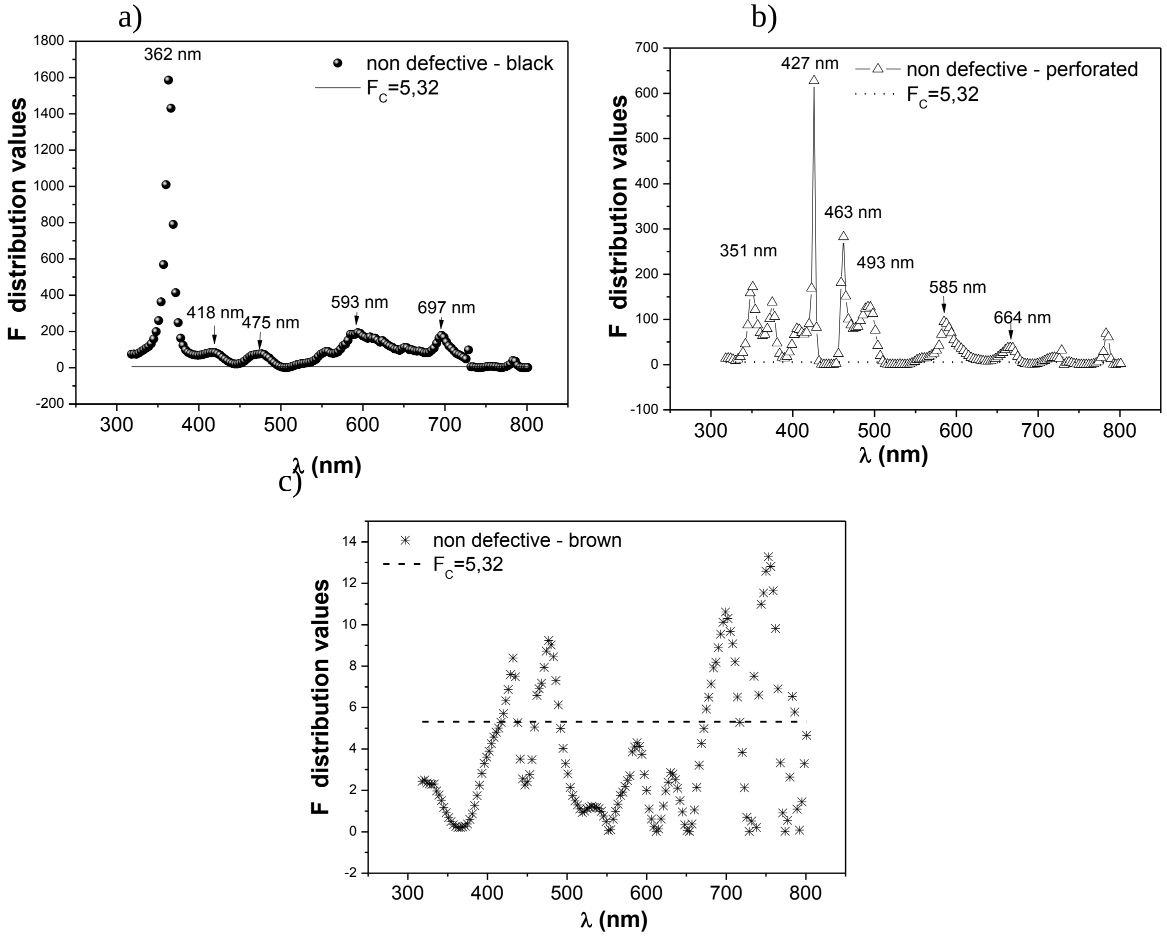

**Fig. 2.14.** F distribution values in function of wavelength obtained of ANOVA study for comparison between roasted and ground coffee spectra first derivative of a) black bean and non-defective bean; b) perforated bean and non-defective bean and c) brown bean and non-defective bean.

### 2.4.3. Chemical composition

Mid-infrared (MIR) measurements were taken adapting a PA cell (MTEC Model 300), which is described in detail elsewhere [38], to a Fourier Transformed Infrared (FTIR) spectrophotometer (Prestige 21 Shimadzu). The sample is located inside a cup and after the cell is hermetically closed helium gas was used in a purge process to reduce water vapor and $CO_2$ in the sample chamber. It ensures reproducibility, signal to noise ratio optimization and minimal spectral interferences. The cell was installed on a base plate of the FTIR in such a manner that the light beam from the lamp impinges on the sample after passing through a quartz window located at the center of the cell.

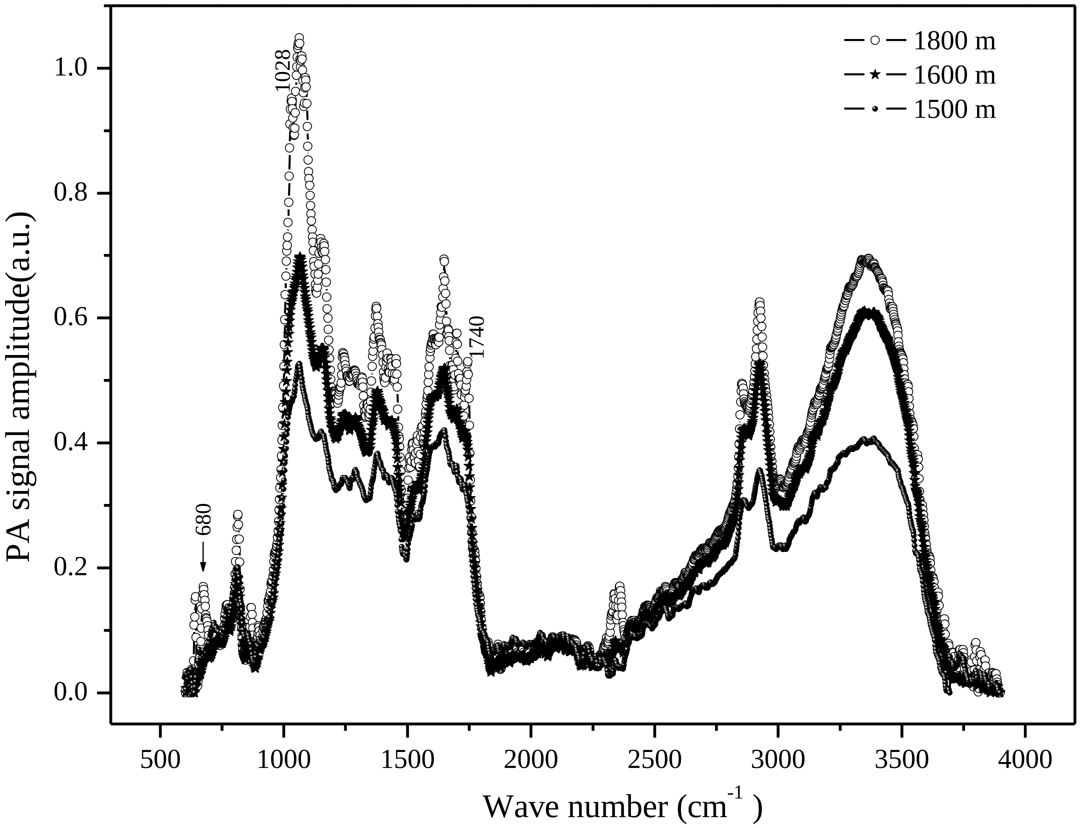

**Fig. 2.15.** Spectra of roasted and ground organic coffee obtained by FTIR-PAS. Coffee samples were collected from different geographical high: 1800, 1600, and 1500 meters over sea level.

MIR PA spectra for organic and conventional roasted and ground coffee are shown in Fig. 2.15 and Fig. 2.16, respectively. Coffee samples were collected from different geographical locations located at 1800, 1600, and 1500 meters over the sea level. Variations in the spectral intensity between 1000 and 1750 cm$^{-1}$ can be seen in Fig. 2.15, while in Fig. 2.16 is showed that the behavior of the conventional coffee spectra is practically constant for samples collected at different altitudes. This spectral range has been selected because it is principally involved to the absorption of caffeine and trigonelline alkaloids. In particular we can see a low-intensity spectral band that appears at 680 cm$^{-1}$ in the organic coffee average spectrum. This absorption center is related to pyridine, chlorogenic acid and malic acid. Thus, chemical composition of organic coffee is more affected by geographical altitude than conventional coffee. This can be due to the whole solar exposition and agrochemicals used in conventional cultivation techniques. Although the chemical composition of coffee is very complex, some compounds have been identified in this chapter (see Table 2.1). We hope that these evidences of drastic changes in composition of organic coffee as a function of geographical altitude can serve as a criterion for origin certification.

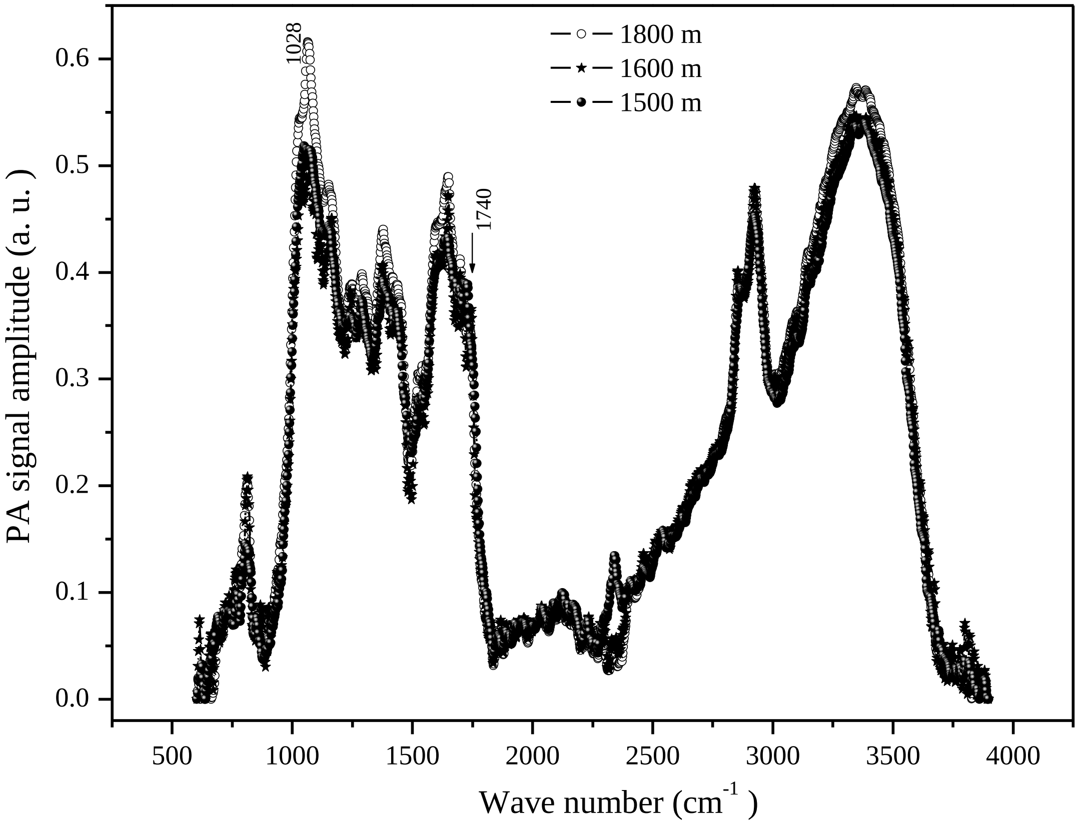

**Fig. 2.16.** Spectra of roasted and ground conventional coffee obtained by FTIR-PAS. Coffee samples were collected from different geographical high: 1800, 1600, and 1500 meters over sea level.

**Table 2.1.** Some compounds identified in organic and conventional roasted and ground coffee. In wave number column appear the location of bands related to components that were marked with X.

| Wave number ($cm^{-1}$) | caffeine | trigonelline | Nicotinic Acid | Pyridine | Glucose | Oxalic acid | Fructose | Quinic Acid | Chlorogenic Acid | Cinnamic Acid | Ferulic Acid f | Caffeic Acid | Citric Acid | Malic Acid | pyruvic Acid | Acetic Acid | pyrazine | Thiazole |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 3740 | | | | X | | | | | | | | | | | | | X | |
| 3356 | | | | | | | | | X | | | | | | | | | |
| 2924 | | | | | X | | | X | | X | X | | | X | | | | |
| 2860 | | | | | X | | | | | X | X | X | | X | | | | |
| 2361 | | | | | | | | | | | | | | X | | | | |
| 1740 | | | | | | | | | | | | | X | X | X | | X | |
| 1695 | X | | X | | | | | | X | | X | | | | | | | |
| 1649 | | X | | | | | | | X | | | | | | | | | |
| 1375 | X | X | | X | X | | X | | X | | X | X | X | X | | | | X |
| 1260 | | | | | | | X | | X | X | | | | X | | | | |
| 1153 | | X | | X | X | | | | X | | X | | X | | X | | X | |
| 1069 | | | | | X | | X | X | | | | | | | X | X | | |
| 812 | | | X | | | | | | X | | X | X | X | | | | | |
| 726 | | | | | | X | | | | X | | | | | | | | |
| 673 | | | | X | | | | | X | | | | | X | | | | |

## 2.5. Effect of biofertilizers on photosynthetic activity of coffee plants

Non-conventional agricultural techniques have been implemented worldwide through the so-called “green revolution” for assuring food to an increasing global population [39]. These practices have altered the nitrogen cycle and eutrophication is one of their most principal consequences. Thus, natural potable water sources and coastal sea zones have been severally affected being converted in sources of nitrous oxide, a greenhouse effect gas [40]. Eutrophication is the most important cause of 245000 square kilometers of sea dead zones [41, 42]. It is hoped that microbial processes can eventually renovate the natural nitrogen cycle. However, environmental friendly agricultural practices are necessary for discontinuing this damage. Biotechnological advances are a potential alternative for agricultural production sustainability using proportional agrochemical products quantities that can be consumed in high percentage by plants, avoiding the use of pesticides. Other strategies can involve natural and mechanical pest control, biodiversity care, and the use of composts.

Bacterial inoculants or “biofertilizers” are fabricated with beneficial microorganisms, which are extracted from the plant root and cultivated under laboratory conditions. Through mutual interaction, these bacteria are specialized for disposing efficiently nutrient absorption to plants and controlling some pests and sickness [43]. This avoids leaching problems and helps to restore the permeability and fertility of super-exploited soils. Biofertilizers use does not compromise the human health and has less economic and environmental cost [44]. However, it is necessary to evaluate the physiological effect of microbial fertilizers application considering local characteristics.

Several authors have proved that photosynthetic activity is affected by fertilization process [45, 46, 47]. It is also known that stomata behavior can change in dependence of the way in which the nutrients are assimilated [48]. So, activity related to photosynthesis can serve for testing biofertilizers. In this chapter an evaluation of the biofertilizers effect on coffee plants photosynthesis and stomata response was done employing the photoacoustic (PA) technique and by means of micrographs respectively. As was described before, the PA technique is based in measuring the pressure changes in a thin layer of gas adjacent to a body radiated with modulated light, which are due to heating by non-radiative de-excitation processes. This effect can be detected as a sound inside a cell that encloses the sample. If in addition, the sample presents photochemical activity, the production of gases contributes to this change. Then, when the PA measurements are resolved in time, it can be used to study photosynthetic activity [49].

Gas exchange methods are the most conventionally utilized for photosynthesis measurements and infrared sensors are commonly applied for carbon dioxide measurement. But the large amount of time that is required for measuring consumed carbon dioxide is the principal disadvantage of this technique [50]. The PA technique becomes cheaper and allows directly oxygen evolution sensing without any gas flux control.

Heat emission and gas evolution or uptake processes generate the PA signal when leafs samples are radiated with periodically modulated light. The most of the time, this evolution corresponds to photosynthetic oxygen [51]. If the photosynthesis process is saturated the PA signal component corresponding to the photothermal (PT) effect is kept while that due to biochemical processes is subtracting from the total signal [52]. The difference between these signals represents the oxygen evolution contribution to the signal.

Samples for selection of beneficial microorganisms from coffee plants rhizosphere were taken from an experimental greenhouse at Quindío University, Armenia- Quindío, Colombia at 1350 m over the sea level. The selection of nitrogen fixing and phosphorus solubilizing bacteria was done using Agar LGI. The cultures were multiplied using submerged fermentations methods with aeration and stirring. Biofertilizers were prepared with a mixture of these bacteria. Concentrations around $10\times10^9$ cfu/ ml were used. The plants of coffee were soaked with a 10 ml biofertilizer per week before sowing.

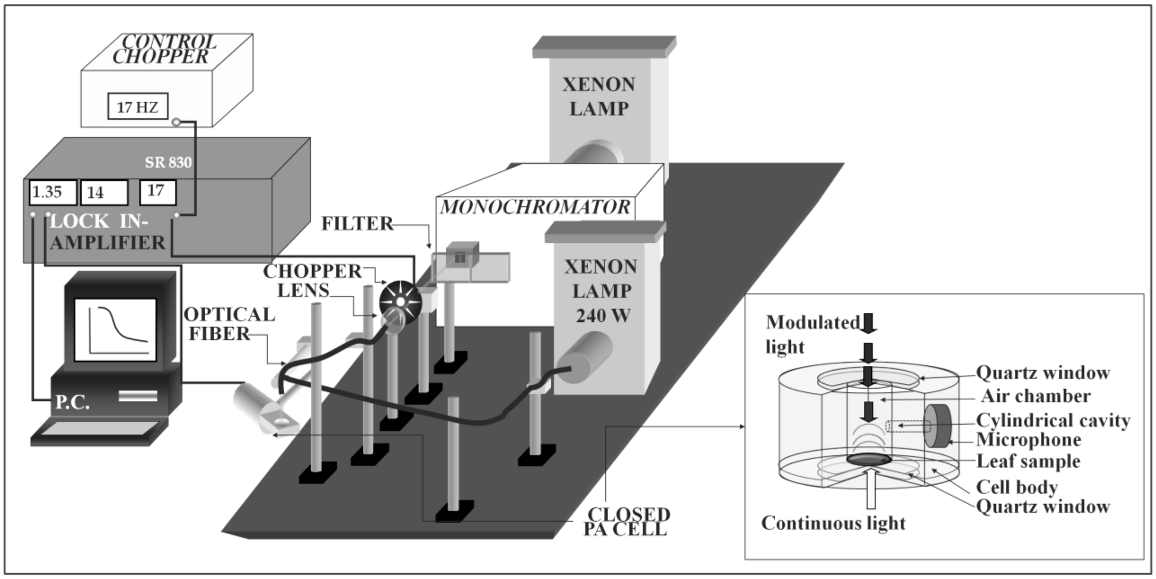

**Fig. 2.17.** Schema of PA system for OER measurements. Continuous light from a halogen lamp was used for saturating photosynthesis process. The leaf closing PA cell is shown in the inset.

Photosynthesis analysis was done in 36 plants of *Coffea arabica* grouped according to: biofertilizer-treated, chemical-treated fertilizer, and water-treated (control group). A PA system illustrated in Fig. 2.17 was used. In this system, a light beam from a Xenon 1000 W lamp (ORIEL) pass through a Jobin Yvon-spex (Triax 190) monochromator and the resulting monochromatic light is amplitude modulated with a mechanical chopper at a 17Hz frequency. The modulated beam is then directed using an optical fiber onto the 4 mm diameter leaf disc sample enclosed in the PA cell. An electret microphone coupled to the cell senses the PA signal, which is then fed to a Lock-in amplifier (SR-830) where it is measured. The PA signal is generated by the periodical heating of the left because of non-radiative de-excitations and oxygen evolution changing. Photosynthesis was saturated with a non-modulated back light from a 240 W Xenon halogen lamp (ORIEL).

The signal amplitude was measured for each sample, and the oxygen evolution rate (OER) was estimated through the decrease percentage in the signal when light was turned on, using equation (1.2) that was described in chapter 1. Fig. 2.18 shows the PA signal as a function of time obtained from leaves of coffee plants grown with different treatments. A non-modulated white light was turned on during 150 seconds, in such a manner that the photobaric component was blocked and the total signal decreases to a value given by the PT component alone. From this curves the OER quantification was done and it is shown in Table 2.2.

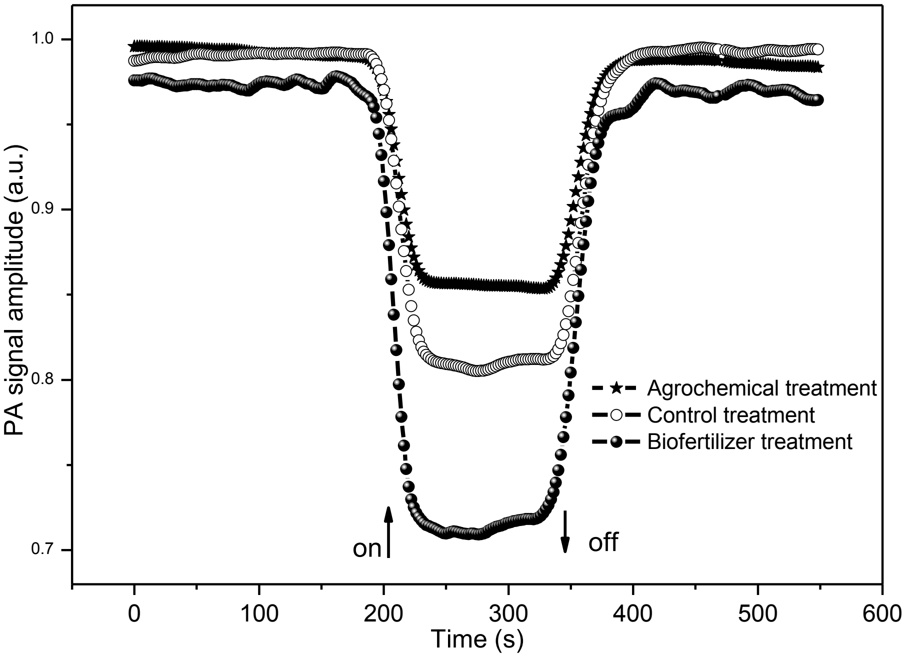

**Fig. 2.18.** PA signal amplitude as a function of time (t). The curves are the average of measurements taken for 12 leaves samples of different *C. arabica.* plants from treatments groups: agrochemicals, biofertilizer and control. The arrows mark the moment when saturation light was turned on (↑) and off (↓).

**Table 2.2.** Stomata total count by area unit (SC/A), opened stomata count (OSC), closed stomata count by area unit (CSC/A), oxygen evolution rate (OER) obtained by PA technique for *C. arabica.* leaves samples.

| **Treatment** | **SC/A ($mm^{-2}$)** | **OSC /A ($mm^{-2}$)** | **CSC/A ($mm^{-2}$)** | **OER (%)** |
|---|---|---|---|---|
| Biofertilizer | 59±1 | 30±2 | 29±2 | 30,0±0,7 |
| Control | 71±2 | 28±2 | 43±2 | 19,6±0,1 |
| Agrochemical | 57±1 | 37±2 | 20±2 | 14,8±0,1 |

From Table 2.2 is possible to observe that the total number of stomas per area unit in agrochemical and biofertilizer treatment group is lower than those corresponding to control-treatment group. Micrographs, as showed in Fig. 2.19 were used for this counting. A quite different behavior was observed for open stomata when leaves are exposed abruptly to white light. The higher value corresponds to the agrochemical group, while biofertilizer and control group present similar values. This result evidences the drastic effect of chemicals on leaves cell structure. On the other hand, the use of biofertilizers increases severally the OER (30%) respecting the control group, while for the agrochemical group this value decreases. Then, it is evident that both photosynthetic activity and stomatal response change according the fertilization treatment. Thus, it is possible that the use of biofertilizers increases photosynthetic activity without producing a strong alteration in the plant morphoanatomy, but further investigations become necessary in order to establish definitive conclusions.

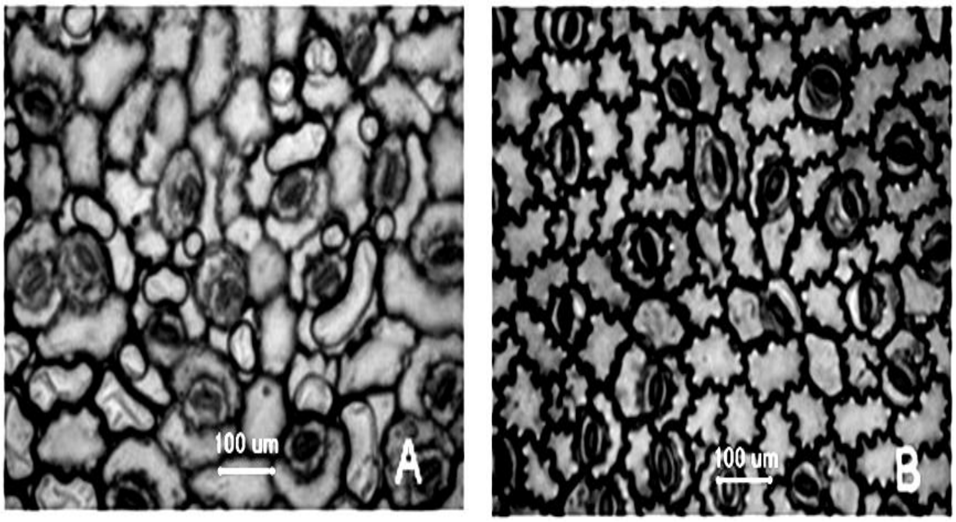

**Fig. 2.19.** Stomata microphotographs for leaves samples from: A) plants with biofertilizer treatment and B) plants with treatment of chemical.

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# CHAPTER 3

## CONVENTIONAL AND ORGANIC RICE

## 3.1 Introduction

Currently, much research has been focused on the development of techniques that assure that there is an added value for agricultural products. Those techniques have been developed to promote the food security from a multidisciplinary approach. Rice is one of the products that have been base for this type of studies. These studies focus on the transformation processes of the rice grain after being roasted and ground. Also the use of organic rice growing techniques and the application of the rice husk in the industry of cements and activated carbons have been taken into consideration by other authors [1].

There are several types and levels of regulation for the production and processing of organic products. There is not a unique norm of organic certification on a global level and some countries have their own regulations. However, the International Federation of Organic Agriculture Movements (IFOAM) and the Codex Alimentarius promote a global certification system. The certifier agency can work according to these general norms, or it may design own norms to guarantee the characteristics of the origin of the products [2].

The rice crops are distributed in tropical and subtropical regions. The 91% of the global production takes place in Asia; America with 5%; Africa with 3%; and the European and Australian continents with 1% also contribute to the worldwide production. This sowing of rice dates from about 10,000 years ago in many humid regions of tropical and subtropical Asia, but it had his maximum development in China. Therefore, procedures have been studied for improving its quality, since rice is one of the main foods for the population of the planet. It contains carbohydrates, fiber, protein, fat, folic acid, iron, calcium, phosphorus, potassium, sodium, among others [3].

In the agricultural production of rice, the seeds of the plant, namely green paddy rice, are harvested. Its industrial processing includes a drying process of green paddy rice (dry paddy), descaling (threshing), and polishing in order to obtain consumption white rice and its derivatives like: divided rice, rice flour, crushed rice, and rice bran [4].

Figure 3. 1 shows rice with (a) and without pericarp (b), respectively. The pericarp or husk can be reddish brown or violet. It is divided into three layers of cells that are the endocarp,

the mesocarp, and the epicarp. The pericarp is known as the lemma or palea according to location in the surface. The rice grain is a caryopsis, which is called brown rice [5].

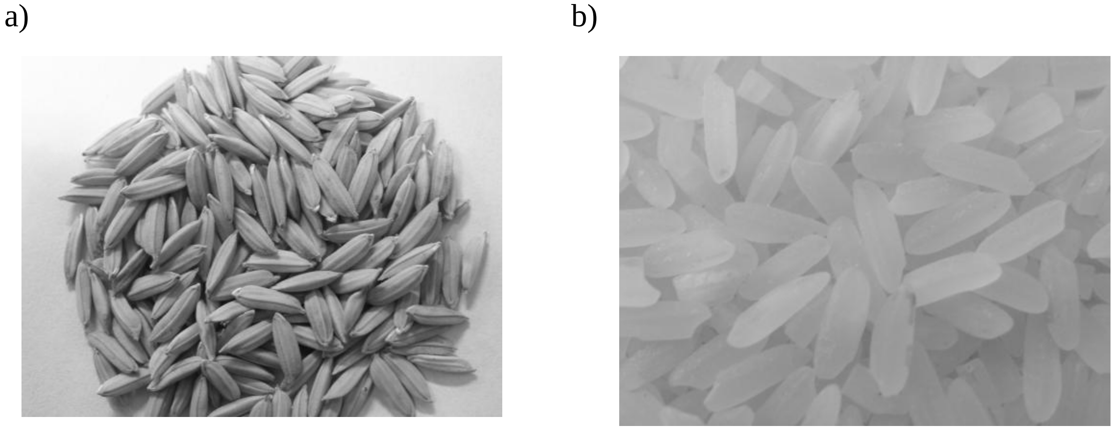

**Fig. 3.1.** Rice seeds: a) with pericarp and b) without pericarp.

Transformation processes (roasted and ground) can be efficiently modeled with information about the thermal diffusivity of the raw material [6, 7]. This parameter is related to the speed of heat transference through a material. Therefore, knowledge of thermal diffusivity allows us to establish appropriate times and temperatures during these processes. On the other hand, the absorption spectrum is considered to be a fingerprint for identifying a material. These patterns allow for the comparison of products that have different characteristics. In this chapter, spectroscopic and thermal diffusivity measurements are reported in order to establish some differences between rice produced with the aid of chemical products and rice produced with the aid of organic fertilizer [8, 9].

### 3.2 Methodology

Paddy rice samples of the *combeima* variety were collected in random way from the municipality of Espinal - Tolima, Colombia, located at 323 m above the sea level, 4° 09' north latitude and 74° 53' to the west; dealt with organic and conventional techniques.

The comparison of the thermophysical parameters, absorption spectra and cellular morphology of the rice husk samples and rice without pericarp according to the type of treatment (synthetic or organic fertilizer) was made in the following way:

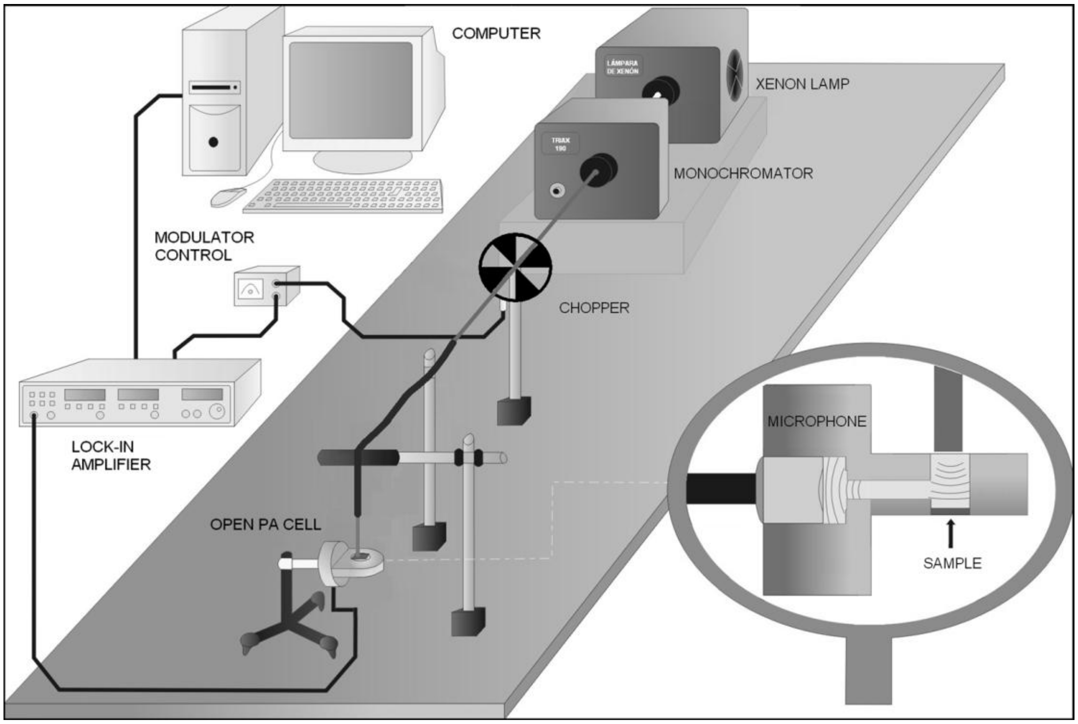

**Fig. 3.2.** Photoacoustic spectroscopy system. The inset shows how the sample is putted into the cavity of a closed cell.

Photoacoustic spectroscopy (PAS) was used for the determination of pigments present in the sample using the experimental setup described in figure 3. 2. The samples were rice grain powder and rice husk. In the first case, rice was roasted and ground; in the second husk was used without any previous process. The setup consists on a 1000 W Xenon lamp (ORIEL_66924), whose white light is focused onto a monochromator (Jobin Yvon - Pex Triax series 190) input slice after passing through a water filter used to eliminate the IR part of the spectrum that can heats the sample in an unwanted way. The output beam is modulated mechanically at a frequency of 17 Hz, and is led by an optical fiber onto the sample, which is placed within the cavity of a closed photoacoustic (PA) cell. Sample heating due to non-radiative de-excitations generates periodical heating that is transferred to a thin layer of air adjacent to the sample, which produces sound as described elsewhere [10]. For the detection of the acoustic signal an electret microphone was used as a transducer, and finally, this signal was measured using a Lock-in Amplifier (SR830), which uses the modulation frequency as a reference for minimizing the noise from other sources.

The analysis of the recorded PA spectra has been performed using both a first derivative criterion and analysis of variance (ANOVA).

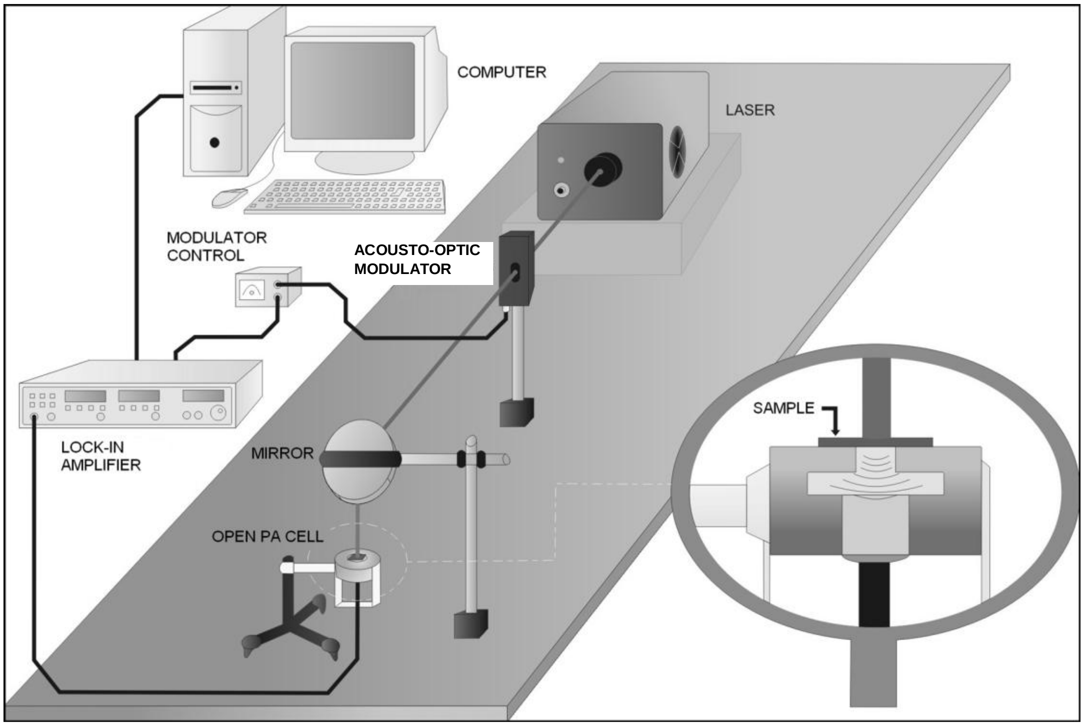

**Fig. 3.3.** System used for the measurement of thermal diffusivity. The inset shows how the sample is putted on an open PA cell (OPC).

For thermal diffusivity measurement the rice grain samples were cut in disk shape of 600μm thickness and 2mm diameter approximately. The experimental setup is shown in figure 3. 3. Light from an Argon laser (Modu-laser) was modulated in intensity through an acousto-optic modulator (HB-Laserkomponenten, Helios 4); the modulated light beam impinges on the sample, which is placed on an open PA cell (OPC), directly upon the orifice of a common electret microphone [10]. When modulated light is absorbed by the sample, its surface is periodically warmed up, and heat is transported to the gas in the cell generating pressure changes that are detected as sound by the microphone. The resultant signal is measured as a function of the modulation frequency using a lock-in amplifier. From the obtained graph the thermal diffusivity can be obtained straightforwardly using the model of Rosencwaig-Gersho [10, 11].

Finally, rice grains morphology observations were made to examine the cellular structure by means of the cross section of 15 randomly selected samples. The cells coloration with a lugol's solution showed the presence of some polysaccharides like starch and glycogen. This observation was done through micrographs that were taken with an optical microscope. A count of the amount of dyed cells was made taking into account the distribution within the epidermis, endosperm, and the central part of the grain.

## 3.3 Results and Discussion

### 3.3.1 Morphology and composition analysis of the rice grain

A lugol's solution was used to dye the rice grain cells and to examine them with the optical microscope. This coloration is frequently used to observe the presence of some polysaccharides like starch and glycogen.

The micrographs of 10 grains showed that the organic rice contains a homogenous distribution of its nutrients. 66.7% of the studied area showed starch presence. From this percentage, 33.3% was in endosperm. This indicates a high amount of polysaccharides in the grain. On the other hand, the samples of conventional rice exhibited heterogeneity in the content of nutrients. In this case, 36.7% of the studied area contained starches in the central part and in the endosperm, and a 26.7% in the epidermis.

In figure 3.4, the darkness gray spots correspond to homogenous distribution regions where there are polysaccharides [4]. These micrographs were taken on transversal cuts of the organic rice samples from *combeima* variety plants. On the other hand, in figure 3.5 a non-homogenous distribution of polysaccharides in the endosperm of conventional rice grains of this same variety is observed.

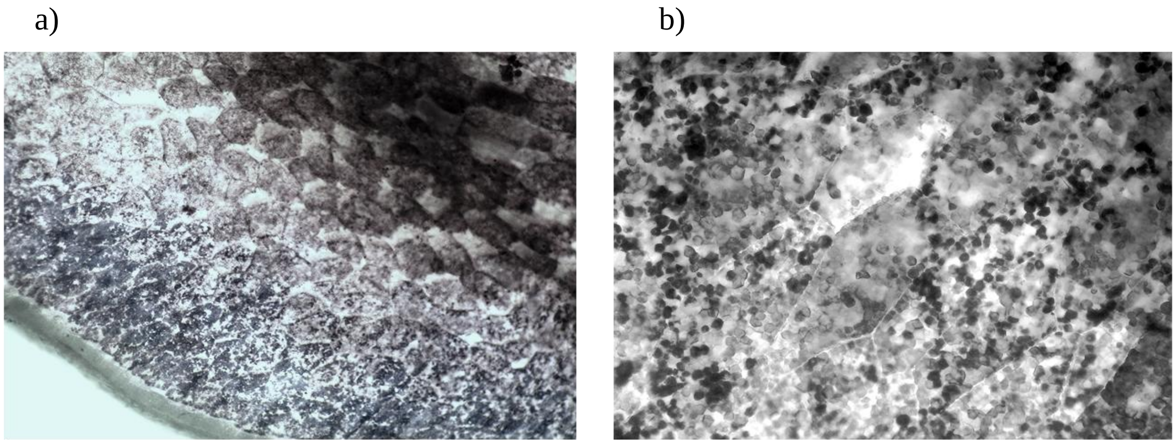

**Fig. 3.4.** Microphotography with a zoom of a) 10X and b) 40X for a sample of organic rice. Zone with darkness gray corresponds to polysaccharides distribution.

**Fig. 3.5.** Microphotography with a zoom of 40X, sample of conventional rice. Zone with darkness gray corresponds to polysaccharides distribution.

### 3.3.2 Thermal diffusivity of the rice husk

The curves of PA signal amplitude as a function of the light modulation frequency were fitted to the Eq. (1.1) and the "cut-off frequency" was taken as a fit parameter. From it the thermal diffusivity is determined if the thickness is well known. In Table 3.1 are shown the average values from measurements performed in seven rice grains.

From these results one can see that the thermal diffusivity of organic rice has a lower value than the one corresponding to conventional rice. This behavior can be related to the nutrients distribution observed and also to possible chemicals inoculation in the grain of plants cultivated in a conventional way.

**Table 3.1.** Values of thermal diffusivity ($\alpha$), cut-off frequency ($f_c$), and sample thickness ($L$) for conventional and organic rice of the *combeima* variety.

| Rice sample | $f_c$ ± 0,02 (Hz) | $L$ ±0,0004 (cm) | $\alpha$ ± 0,001 ($cm^2/s$) |
|---|---|---|---|
| Organic | 0,58 | 0,0615 | 0,007 |
| Conventional | 0,91 | 0,0610 | 0,011 |

### 3.3.2 Spectroscopic study of the rice husk

A spectroscopic study for rice husk samples from conventional and organic crop was made in order to study possible pigmentation changes. The spectra were the result of measuring average for ten samples, which were chosen randomly. The differences in the form of the spectra were quantified using the first derivative criterion and ANOVA [12].

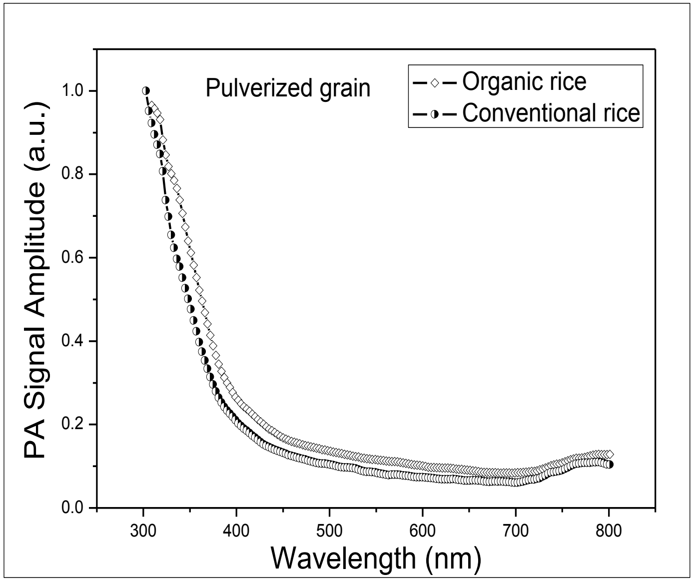

**Fig. 3.6.** PA absorption spectra for samples of conventional and organic rice, after being ground. Empty squares symbols correspond to organic sample, while open circles correspond to a conventional sample.

The spectra of ground rice from roasted and non-roasted samples are showed in figures 3.6 and 3.7, respectively. In these results, significant differences are not observed in the light absorption behavior according to the type of the applied fertilizer in the cultivation. This indicates that the samples have similar pigments distribution.

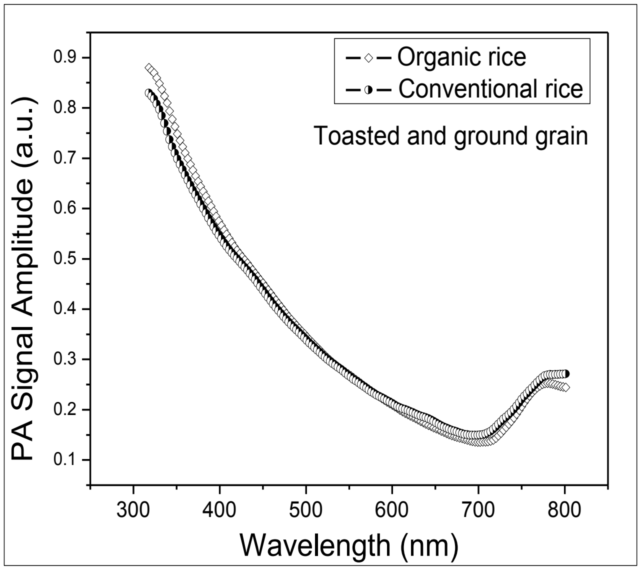

**Fig. 3.7.** PA absorption spectra for samples of conventional and organic rice after roasting and ground process. Empty squares and open circles correspond to organic and conventional sample respectively.

The resolved-wavelength PA signal amplitudes in figure 3.8 correspond to the pericarp (husk) of the organic and conventional sample. These spectra show a wide absorption center situated between 320 and 380 nm that is related to the capsaicin presence; and other one, between 380 and 458 nm corresponding to the presence of β-carotene, lutein, violaxanthin and neoxanthin, with a maximum intensity value at 411 nm attributed to the absorption of the capsorubin pigment. A considerable difference between the first derivative of both spectra in the ranges 320-430 nm and 450-670 nm can be seen in figure 3.9. The absorption center in the infrared region above 720 nm can be related to the morphologic structure of the sample. This behavior is the same for the two considered kinds of samples.

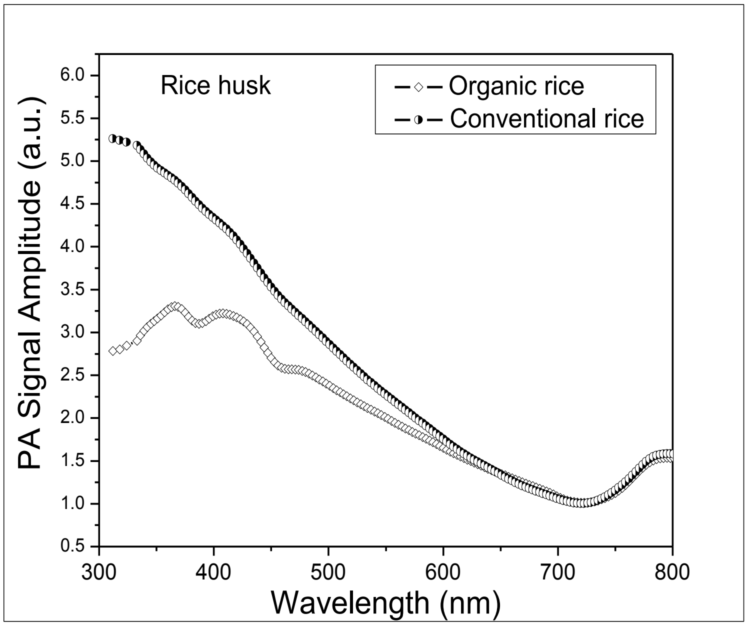

**Fig. 3.8.** PA absorption spectra for husk samples of conventional and organic rice. Empty squares correspond to organic sample, while semi-full circles correspond to conventional sample.

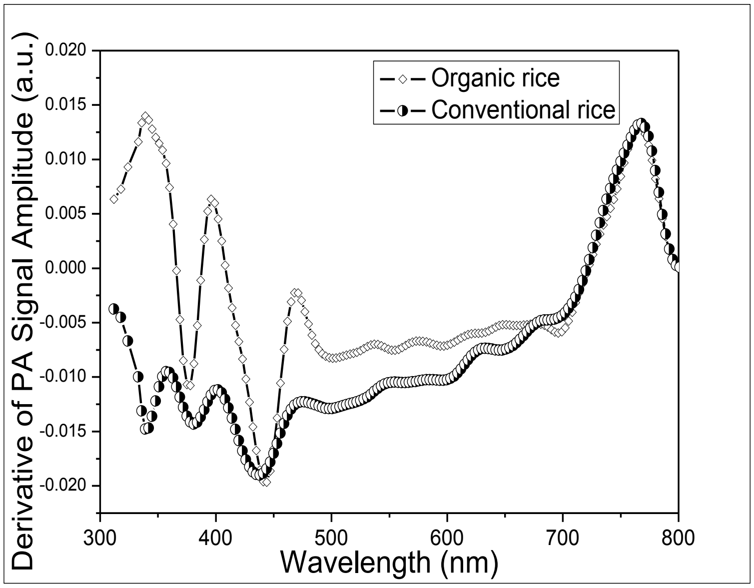

**Fig. 3.9.** Derivatives of PA absorption spectra amplitude. Empty squares correspond to organic sample, while semi-full circles correspond to conventional sample.

In figure 3.10, the difference between the spectra for the organic and conventional samples of rice husk is evidenced through subtraction of PA signal amplitude derivatives. Maxima in this curve indicate the wavelength values for which light absorption by pigments is better resolved for organic rice husk. It is probably due to a less homogenous distribution of pigments in the conventional sample.

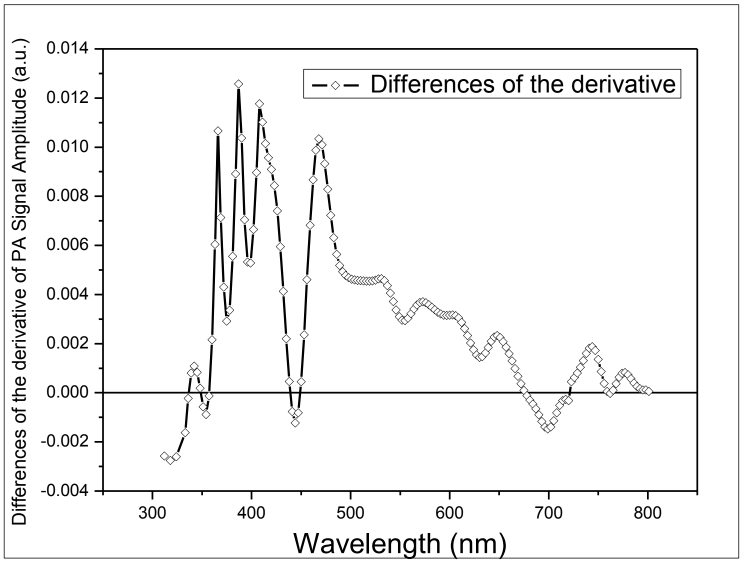

**Fig. 3.10.** Subtraction of the PA absorption spectra derivative for husk samples of conventional and organic rice.

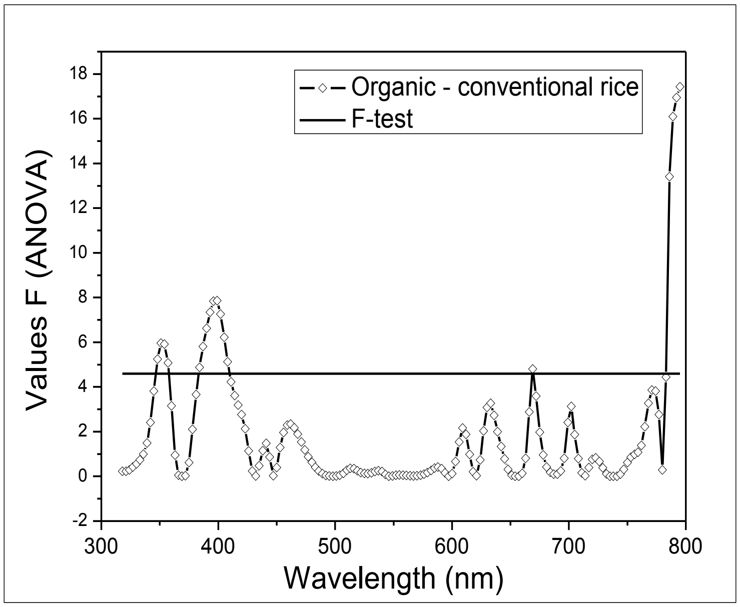

**Fig. 3.11.** Analysis of variance (ANOVA) between PA spectra of organic and conventional rice husk.

In figure 3.11 the ANOVA for the rice husk spectra shows significant differences between the organic and conventional treatments. The F-test was 4.6 according to Fisher distribution with a significance level of 5% [12]. This curve corresponds to the theoretical determined F for each wavelength. The ranges 348 to 356 nm, 383 to 408 nm and 786 to 795 nm have a theoretical F greater than that of the F-test. Therefore, the null hypothesis is rejected, assuring a significant difference in these wavelength ranges.

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# CHAPTER 4

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## EFFECT OF *Azospirillum brasilense* AND *Burkholderia unamae* BACTERIA ON PHOTOSYNTHETIC ACTIVITY OF MAIZE

## 4.1. Introduction

Symbiotic relation of mutualism between several terrestrial plants and some soils microorganisms has become the subject of great scientific interest because dramatic effects of weather changing caused by conventional agricultural practices have been documented in the last years [1]. Nowadays, bacterial inoculants are a potential alternative for agricultural production sustainability without the use of chemicals products [2, 3, 4].

Although plants growth promoting bacteria are well characterized, it is convenient to evaluate *in situ* their effects on the plants. Effects of *Azospirillum*–plant associations have been observed in other works as a contribution of microbial biological nitrogen fixation to the plant through physiological responses examination. Morphological and physiological changes of the inoculated plant roots with this bacterium lead to an enhancement of water and mineral uptake [5]. On the other hand, the nitrogen-fixing *Burkholderia* species has showed a great potential for agricultural applications as plant growth-promoting rhizobacteria, in rhizoremediation, phytoremediation, and pathogens control [6, 7]. However, the effects of these specific bacteria strains on maize plants photosynthetic activity are not well known [8].

Beneficial bacteria are naturally occurring soil microorganisms that colonize roots and promote plant growth, improving the plant physiological status (mineral nutrition, water availability, photosynthesis etc.). It is of great importance for agriculture practice to study the physiological effects of microbial fertilizers and compare plant productivity in economically important crops, such as maize, after treatment with biofertilizers and N fertilizers used in conventional agriculture practice. Root inoculation with beneficial bacteria is certainly a relevant and tested technique for enhancing crop yield. Evidence about the positive effect of bacterial inoculums on plants, including maize physiology, has been reviewed by Fuentes-Ramirez and Caballero-Mellado [9, 10]. In these works, the effectiveness of nitrogen fixing bacteria on the plant production was determined and analysis on the plant physiological status (for example mineral nutrition, in particular N, biomass production, chlorophyll content, some morphological parameters, etc) has been showed. Because photosynthesis and respiration determine in great extent plant productivity, the main part of the experiment described here has been focused in photosynthetic measurements and effects on thermal diffusivity of maize leaves.

To study photosynthesis the majority of the authors use conventional techniques such as manometric, electrochemical sensors, carbon dioxide isotopes, fluorescence and gas exchange. This last method is the most commonly utilized and infrared sensors are the most frequently applied for $CO_2$ measurement in this category. This technique has the handicap that it limits the amount of time that is required for measuring consumed carbon dioxide [11]. The photoacoustic (PA) technique is an alternative method that has been used elsewhere [12, 13] for this purpose. It is based mainly in the detection of the pressure oscillations in a thin layer of gas adjacent to a sample exited with modulated light [14]. If in addition, the sample presents photochemical activity, the production of gases contributes to these oscillations that can be detected with a microphone enclosed with a sample in a measuring cell. In the case of green plants samples one can study their photosynthetic activity using time resolved PA measurements, as described elsewhere [15, 16]. This method results relatively simple when it is compared to the conventional one as it senses oxygen evolution directly without any gas flux control.

Heat emission due to photothermal (PT) effect, and gas evolution (Photobaric, PB, effect), generate the PA signal when leaves samples are radiated with periodically modulated light. The most of the time the later mechanism corresponds to emitted photosynthetic oxygen. If the photosynthesis process is saturated, the component corresponding to the PT effect remains unaltered, while that one due to the biochemical process is subtracting from the total signal. The difference between these signals represents the oxygen evolution contribution to the signal, determining the so-called Oxygen Evolution Rate (OER).

In this chapter, the effect of bacterial symbiosis on the photosynthetic process of maize plants has been examined *in vivo* and *in situ* using PA based OER measurements for *Azospirillum brasilense* and *Burkholderia unamae* bacteria. Although several works have been reported that study the influence of mycorrhiza on photosynthesis in plants such as tomato and maize using the so-called open PA cell (OPC) technique [17, 18, 19], in this chapter the i*n situ* and *in vivo* measurements of photosynthesis activity were done using a closed PA cell, which avoid the phothermal contribution from the microphone metalized electret diaphragm used in OPC when light transmitted through the leaf is absorbed by it [20].

## 4.2. Experimental

### *4.2.1. Samples preparation*

*Zea mays* seeds were inoculated before sowing with two kind of free nitrogen fixing for the previously well characterized bacteria: *Azospirillum brasilense* (strain Cd) and *Burkholderia unamae* (strain MT1-641$^T$) using the procedures described in detail by Day *et al* [21] and by Caballero-Mellado *et al* [22] respectively. *A. brasilense* and *B. unamae* were grown during 18 hours in Nfb and BSE medium, in that order. The seeds were inoculated with 1 ml of this suspension, which was adjusted to an optical density of 0.3 for the first solution and 0.2 for the second one. The seeds were sown in an inert substrate of sterilized river sand located in 500 ml plastic pots under greenhouse conditions (mean temperature of 16 $^0$C of and 70 % of relative humidity).

Photosynthesis analysis was done with 15 plants of maize grown from these seeds, which were grouped according to three treatments using two sets of five seedlings inoculated with *A. brasilense* and *B. unamae* respectively, and a not treated control group. In all cases, 100 ml of the fertilizer in solution (4g/l) was applied per week to each sample in the same proportion. Measurements were done daily in three plants of each group during 6 weeks at summer season after 20 days of sowing. The temperature during the experiments was 23$^0$C.

### *4.2.2. Photoacoustic measurements*

A homemade PA spectroscopy system shown schematically in the Fig. 4 1 was used for monitoring the photosynthetic activity. A 500 W white light beam from a Xenon arc lamp (ORIEL 66924-1000 W) is intensity modulated with a mechanical chopper (Oriel 75159) at a given frequency after passing through a monochromator (ORIEL Cornerstone 130 1/8 m), which was used for collimating the white light only. The collimated and modulated beam is focused using a liquid light-guide (Oriel LLG212) onto the leaf sample that closes hermetically a conventional PA cell as shown in the inset of the figure. The impinging light beam power density was 5 mW/cm$^2$. An electret microphone coupled to the cell recorded the PA signal, which was measured by a Lock in amplifier (SR850).

The whole system is controlled by a personal computer that allows automatic data recording and processing.

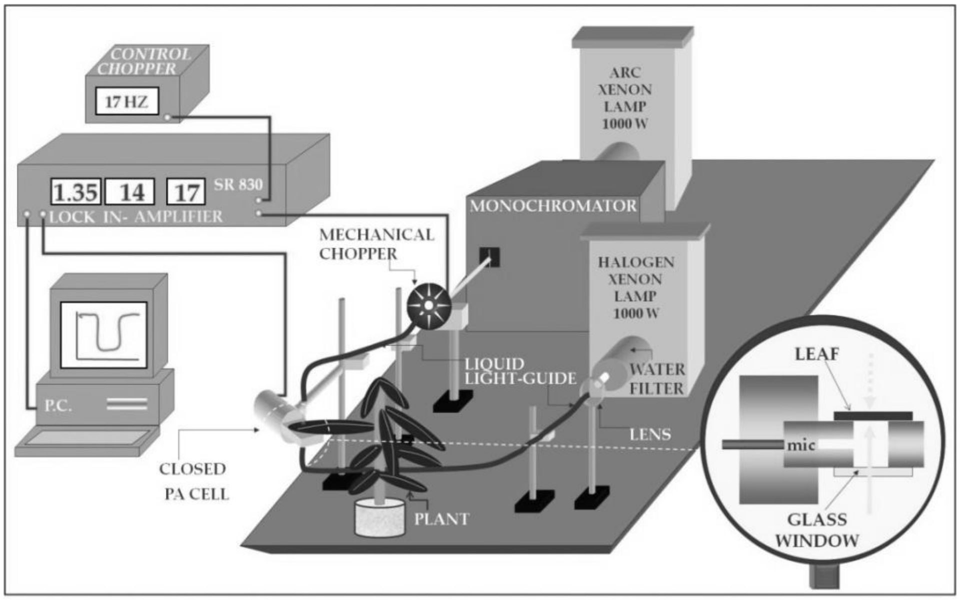

**Fig. 4.1.** Schema of the PA system used for OER measurements showing details of the PA cell and sample´s mounting configuration. The leaf closing PA cell is shown in the inset.

The PA signal is generated by periodic heating of the leaf and by oxygen evolution, as described before. Photosynthesis was saturated using a non-modulated white light beam from a 400 W Xenon halogen lamp (ORIEL 66925-1000 W) impinging on the rear surface of the leaf in contact with the PA gas chamber after passing through a transparent glass window that also closes the PA cell (see figure’s inset). A water filter has been introduced to absorb the infrared portion of the spectrum thus minimizing undesirable heating. This background light closes the reaction centers of the leaf in such a manner that only the modulated incident light is converted into heat. This is because in this moment photosynthesis takes place continuously and is not affected by the pulsed radiation. Then, the PA signal corresponds only to that coming from a photosynthetically inhibited leaf.

## 4.3. Results and discussion

Figure 4.2 shows typical PA signal amplitude as a function of time as obtained from leaves of maize plants grown with the different treatments. The modulation frequency was varied between 10 and 37 Hz. At each frequency the total signal amplitude decreases its value (*A*) to that due to the PT component along (*A´*) when the back non-modulated white light was turned on. Thus, the information obtained from the curve was used for monitoring directly the OER, which was calculated using Eq. (1.2) from chapter 1. The mean values of several independent measurements are shown in Table 4.1.

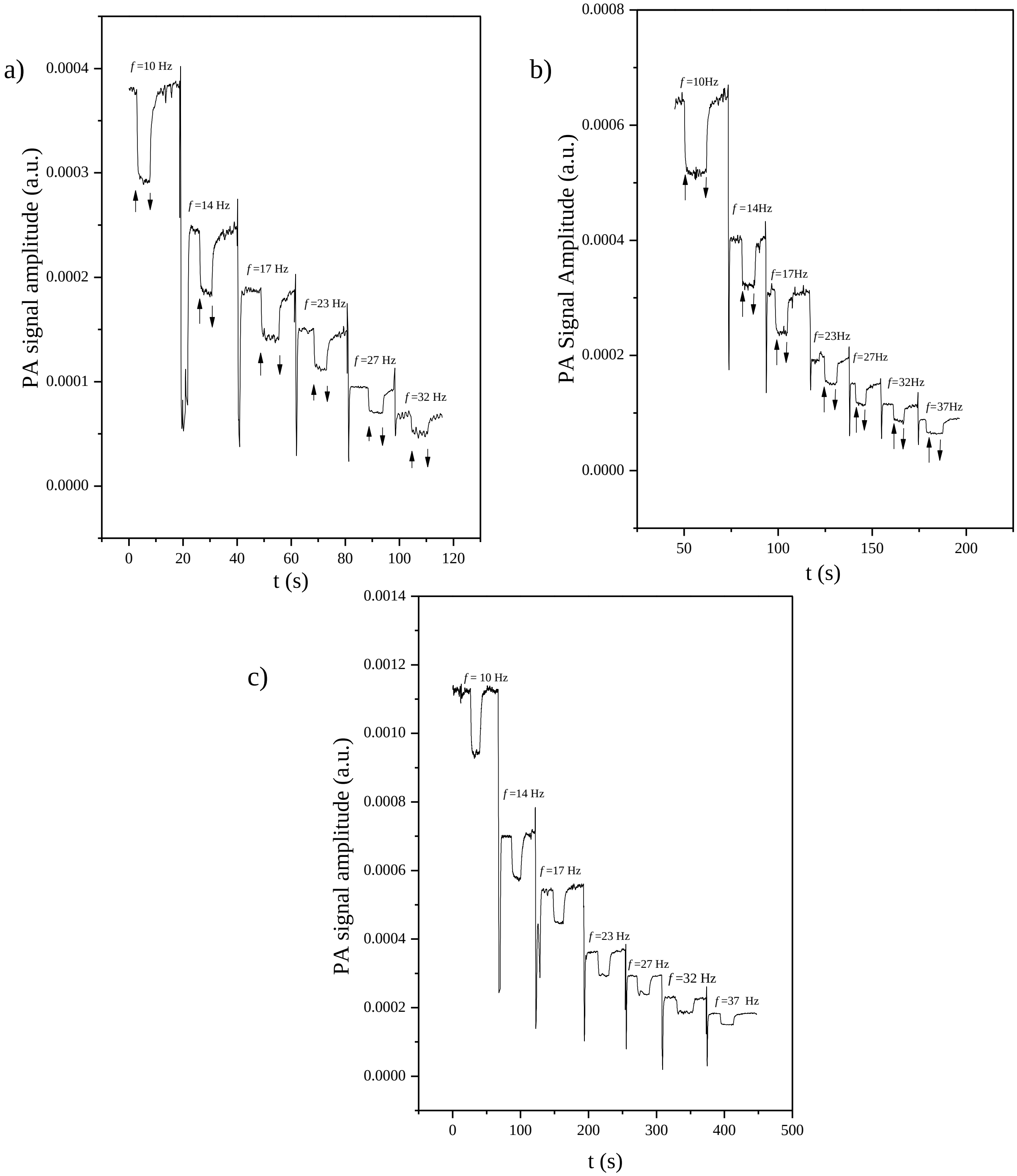

**Fig. 4.2.** PA signal amplitude as a function of time (t) for a leaf of maize plant from (a) control group (b) seeds inoculated with *B. unamae* and (c) seeds inoculated with *A. brasilense*. The arrows indicate the moment when oxygen evolution saturating light was turned on (↑) and off (↓).

For the interpretation of these results thermal diffusivity and oxygen diffusion coefficient have been estimated as well. First, to determine the thermal diffusivity, we measured the PA signal as a function of $f$ under oxygen evolution saturation conditions, i.e. we measure the signal S´. In the Fig. 4.3 typical graphs of the logarithm of the $A´*f$ product as a function of the square root of the modulation frequency are shown. The solid lines are the results of the best linear least squares fits according to Eq. (1.1) from which the value of $\alpha$ was calculated from the slope of the graph, namely $L\ (\pi/\alpha)^{1/2}$ [23], using a measured leaf thickness of $L$= 200 µm.

Then we repeat the same procedure without saturating the PB component. According to Eq. (1.3) in chapter 1, it is possible to estimate the term $(1/D_0)^{1/2} + (1/\alpha)^{1/2}$ from the slope of the $\ln(R)$ vs. $f^{1/2}$ plot (Fig. 4.4), from which we can calculate $D_0$ using the previously obtained value of $\alpha$ and a typical literature reported value of $l$=1µm [24]. The values of both the mass diffusion coefficient and the thermal diffusivity for the three treatments considered in this chapter are reported in Table 4.1.

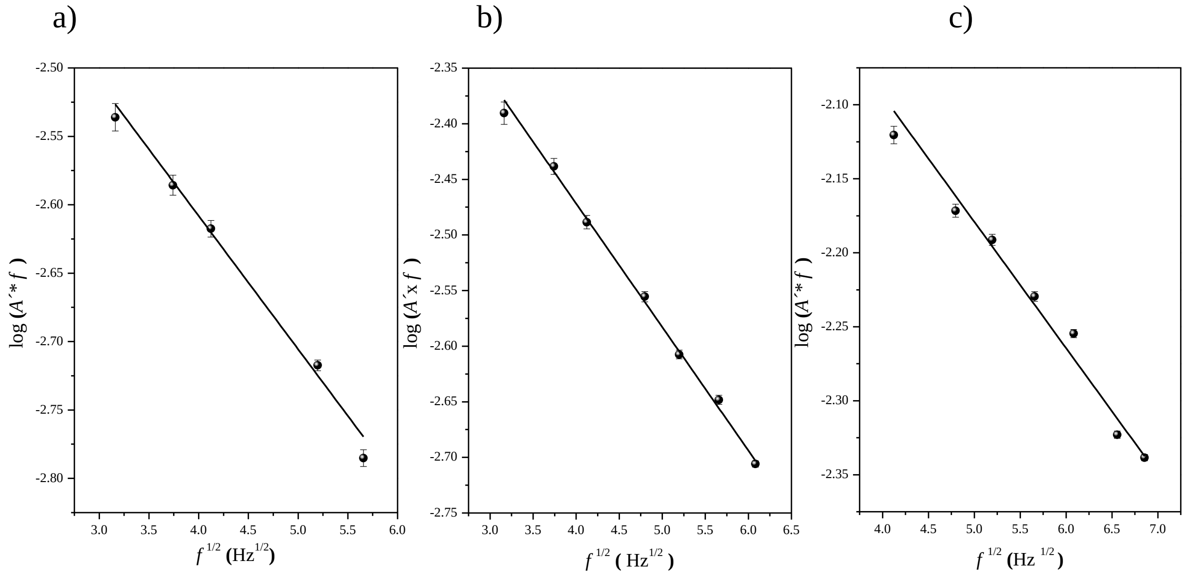

**Fig. 4.3** Frequency dependence of the photoacoustic signal in leaves of maize (a) non-inoculated, (b) inoculated with *B. unamae* and (c) inoculated with *A. brasilense*. The logarithm of the $A´*f$ product is plotted as a function of the square root of the modulation frequency $f$. The solid line corresponds to the best least squares linear fit according to the well-known predictions of the RG model.

From Table 4.1 it is possible to observe that the isolated use of *B. unamae* and *A. brasilense* on maize plants decrease the estimated values of the oxygen diffusion coefficient and of the thermal diffusivity respecting the control group (without bacteria inoculation). The thermal diffusivity behavior suggests changes in the leaf internal composition due to bacteria inoculation. These changes can be related with increasing viscosity that causes resistance to oxygen diffusion, as suggested by the reduction in the oxygen evolution rate values respecting those of the control group.

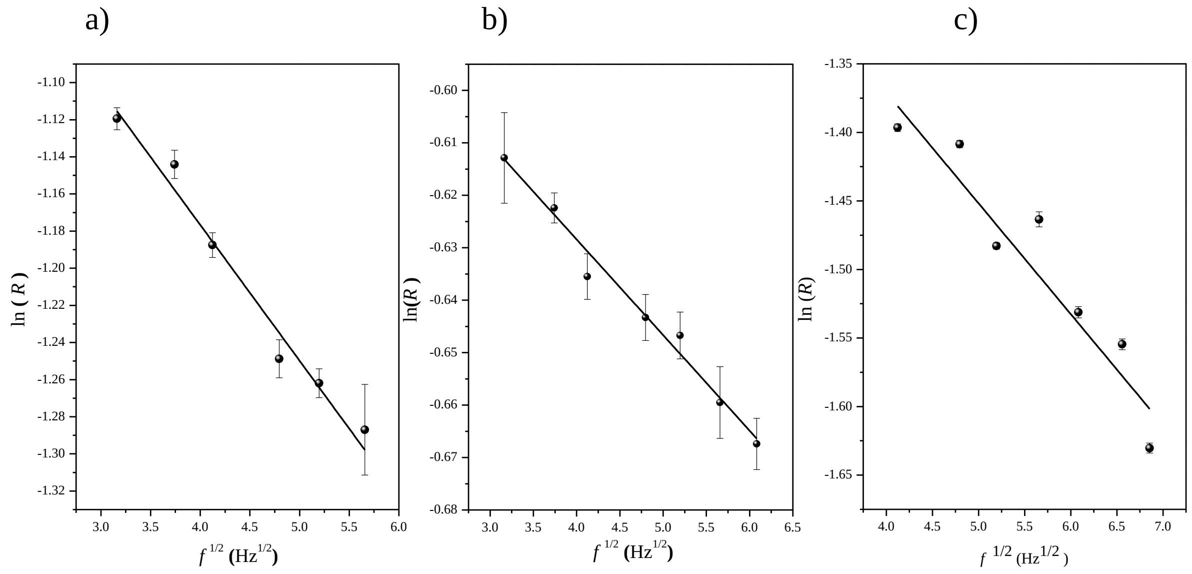

**Fig. 4.4** Frequency dependence of the photoacoustic signal in leaves of maize plants (a) non-inoculated, (b) inoculated with *B. unamae* and (c) inoculated with *A. brasilense*. The logarithm of the amplitude of $R$ is plotted as a function of the square root of the modulation frequency $f$. The solid line corresponds to the best least squares linear fit according to eq. (1.1) from chapter 1.

**Table 4.1.** Mean OER values, thermal diffusivity ($\alpha$**)** and oxygen diffusion coefficient ($D_o$) for the different groups of treated maize plants.

| Treatment | $\alpha$ (cm$^2$/s) | OER (%) | $D_o$ ($\times10^{-6}$cm$^2$/s) |
|---|---|---|---|
| *A. brasilense* | 0.025±0.002 | 21±1 | 4.2±0.7 |
| *B. unamae* | 0.034±0.002 | 18± 1 | 4.2±0.5 |
| Control | 0.044±0.005 | 23±1 | 5.5±0.6 |

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# CHAPTER 5

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## THERMAL DIFFUSIVITY BEHAVIOR OF *Guadua angustifolia* KUNTH AS A FUNCTION OF CULM ZONE AND MOISTURE CONTENT

## 5.1. Introduction

Bamboo is a cheap and fast-grown forest resource with important physical and mechanical properties. Today the bamboo market has acquired great importance. Although many kinds of wooden products are not used anymore with the spread of cheaper artificial materials, bamboo has still great potential for replacing wood in several applications. For example flexible bamboo strips are widely used in bent parts of traditional handiwork, even in the modern age of plastics. Bamboo is also used in many communities worldwide for making various daily use objects, such as cooking items, and also as a building material, applications in which knowledge of heat transfer behavior is of great importance. For many applications a drying process of the material is necessary, and to perform it in an efficient way accordingly to industrial requirements, accurate knowledge of thermal properties (e.g. thermal diffusivity, $\alpha$) and their dependence with moisture is of great importance. The determination of these properties is also important because they depend strongly on other material characteristics such as structure, composition, moisture, porosity, morphology, etc. Thus in order to support the potential of bamboo as a useful industrial resource further research towards its thermal characterization becomes impetuous. It is important to notice that while mechanical properties characterization of Bamboos has been performed by several authors [1, 2, 3], reports on their thermal properties are scarce [4].

Thermal diffusivity is measured using non-stationary or dynamic methods [5]. Because

$$k = \alpha C , \tag{5.1}$$

where $C$ is the specific (volume) heat capacity, knowledge of this last parameter is necessary if the thermal conductivity is to be obtained as well. But fortunately $C$ is nearly a constant parameter for solids being less sensitive to impurities and structure of materials, and comparatively independent of temperature above the Debye temperature, than thermal conductivity and diffusivity. This almost constant value of $C$ can be explained by taking into account its definition as the product of the density ( $\rho$) and the specific heat ($c$). The specific heat is defined as the change in the internal energy per unit of temperature change; thus, if the density of a solid increases (or decreases) the solid can store less (or more) energy. Therefore, as the density increases, the specific heat must decrease and then the product $C = \rho c$ stays constant, so that according to Eq. (5.1) the behavior of the thermal conductivity becomes similar to that of the diffusivity.

Among the dynamic methods for thermal diffusivity measurements the so-called photothermal techniques [6], and in particular the photoacoustic ones [7], have demonstrated their usefulness, having advantages respecting other methods because they are cheaper, non-invasive (they do not require special samples preparation for their measurement, they are measured as they are), the temperature variations involved are so small that they not modify samples properties during the measurement process, and the required sample´s volumes are relative small (typical samples dimensions are in the order of 1cm×1cm×0.05cm). Moreover the physical-mathematical formalisms behind these techniques are relatively straightforwardly allowing easily interpretation of the experimental results.

Due to the above mentioned constancy of $C$ and the definition (5.1), in a plot of thermal conductivity versus thermal diffusivity solid materials typically fall along a line. Debye´s theory for specific heat shows that the slope of this line is about $C=\rho c \approx 3\times10^{6}$ J/m$^3$K at room temperature, if the volume occupied per atom in a solid is taken as about $1.4\times10^{-29}$ m$^3$, an almost common value that is used by many authors. But deviations from this value could be expected for some materials due to several reasons, among them: i- heat conduction can be limited partially by the gas entrapped in the porosity; ii- heat fluxes through parallel arrangements of cylindrical layers and through embedded regions from different materials composing the plant that can modify strongly their effective thermal properties values [8], iii- high values of the Debye temperature so that the classical approximation considered above does not work anymore. In many cases the methods used for measurement of specific heat capacity involve temperatures that can modify sample´s thermal parameters during measurement, particularly in the vicinity of phase transitions and structural changes. Thus, in order to account for the specific heat capacity we resorted in this paper to its calculation using the well-known relationship

$$\varepsilon = C\,\alpha^{1/2} \qquad (5.2)$$

where $\varepsilon$ is the thermal effusivity. For the role of this parameter in heat transport phenomena the interested reader can be referred to the work of Marín [9]. It will be also determined here by the photoacoustic technique using a method based in the effective medium theory that uses the well-known analogy between thermal and electrical phenomena.

Around 1200 bamboo species have been identified in the world [10] and the use of this plant has a very long history, being one of the oldest building materials used by humans [11]. One of the most important species in Central and South America is *Guadua angustifolia* (*Guadua* for short). In this paper we will study, using the PA technique aided with Scanning Electron Microscopy (SEM), the influence of moisture during the drying process on the thermal diffusivity of samples cut from three different culm zones of *Guadua* plants.

## 5.2. Material and methods

### 5. 2.1. Samples

**Fig. 5.1.** The *Guadua* culm was divided in three zones through its longitude: bottom, middle and top.

Measurements were done in 600 μm thick and 1 cm diameter disc shaped samples of *Guadua* cut from the top, middle and bottom culm zones of the plant (Fig. 5.1). Different moistures values were obtained by a drying process using a Metler-Toledo balance with an internal heating system. Changes in the moisture content of the samples were avoided by storing them in a hermetically closed box with humidity and temperature control. The moisture values were normalized to the initial ones measured immediately after the sample was cut.

### 5.2.2. Photoacoustic measurements

***Thermal diffusivity***

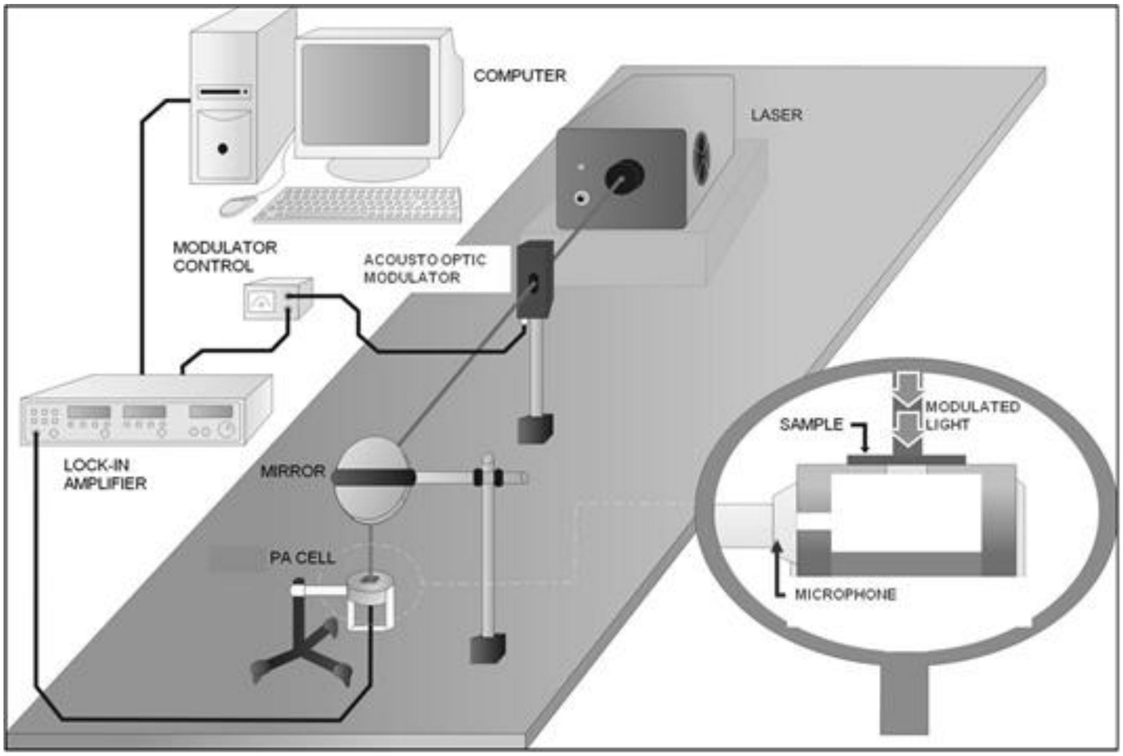

**Fig. 5.2.** Schematic view of the PA system for thermal diffusivity measurements. The inset shows the used open PA cell described in the text.

Thermal diffusivity measurements were performed using a homemade apparatus showed schematically in Fig. 5.2. Modulated light energy absorption takes place at the surface of the sample that closes the air filled chamber of a photoacoustic cell (see inset of the figure). The light source was a 514 nm Argon ion Laser (Modu-laser, Stellar-Pro ML/150) with a power of 100mW. Intensity modulation of the laser light beam has been achieved by means of an acousto-optic modulator (HB-Laserkomponenten, Helios Blanking-unit system 4). The periodically generated heat is transmitted through the sample to the air inside the cell inducing pressure oscillations that are detected by an electret microphone (RadioShack 270-092C) already enclosed in the chamber. The amplitude of the microphone signal is measured as a function of the modulation frequency, *f*, using a Lock-in Amplifier (Stanford Research, SR-830).

From the fit of the experimental data to equation (1.1) in chapter 1, the sample's thermal diffusivity can be determined in a straightforwardly way.

### *Thermal effusivity*

Using a model based in the well-known analogy between electrical and thermal phenomena, the effective thermal diffusivity of a two layer series system can be defined as [12].

$$\alpha_e = \frac{1}{\frac{x^2}{\alpha_1} + \frac{(1-x)^2}{\alpha_2} + x(1-x)\left(\frac{\lambda}{\alpha_1} + \frac{1}{\lambda\alpha_2}\right)}, \qquad (5.3)$$

where $x = l_1/(l_1 + l_2)$ is the ratio of the thicknesses of the involved materials (subindices 1 and 2 refer to each of them) and $\lambda = k_1/k_2$, is the ratio of their conductivities. Using Eq. (5.1) this ratio can be expressed as

$$\lambda = \frac{k_1}{\varepsilon_2\sqrt{\alpha_2}} \qquad (5.4)$$

The effective thermal diffusivity of a sample composed by the investigated bamboo (material 2 in our model, for which thermal diffusivity, $\alpha_2$, is known from a previous measurement using the procedure described above) and a sample of another material (a reference sample 1 with well-known thermal properties $\alpha_1$ and $k_1$) attached to it can be measured as described in the preceding subsection. If $x$ is known then the parameter $\lambda$ can be determined using Eq. (5.3). From it the thermal effusivity, $\varepsilon_2$, can be calculated straightforwardly using Eq. (5.4).

### 5.2.3. Scanning electron microscopy

Micrographs of longitudinal and transversal cuts of samples were taken using an Scanning Electron Microscope (SEM) JEOL JSM-6390LV at amplification of 500x and acceleration voltage of 20kV. This kind of Microscope has been selected because it allows measurements under low vacuum conditions so that water evaporation from the samples is minimized [13].

## 5.3. Results and discussion

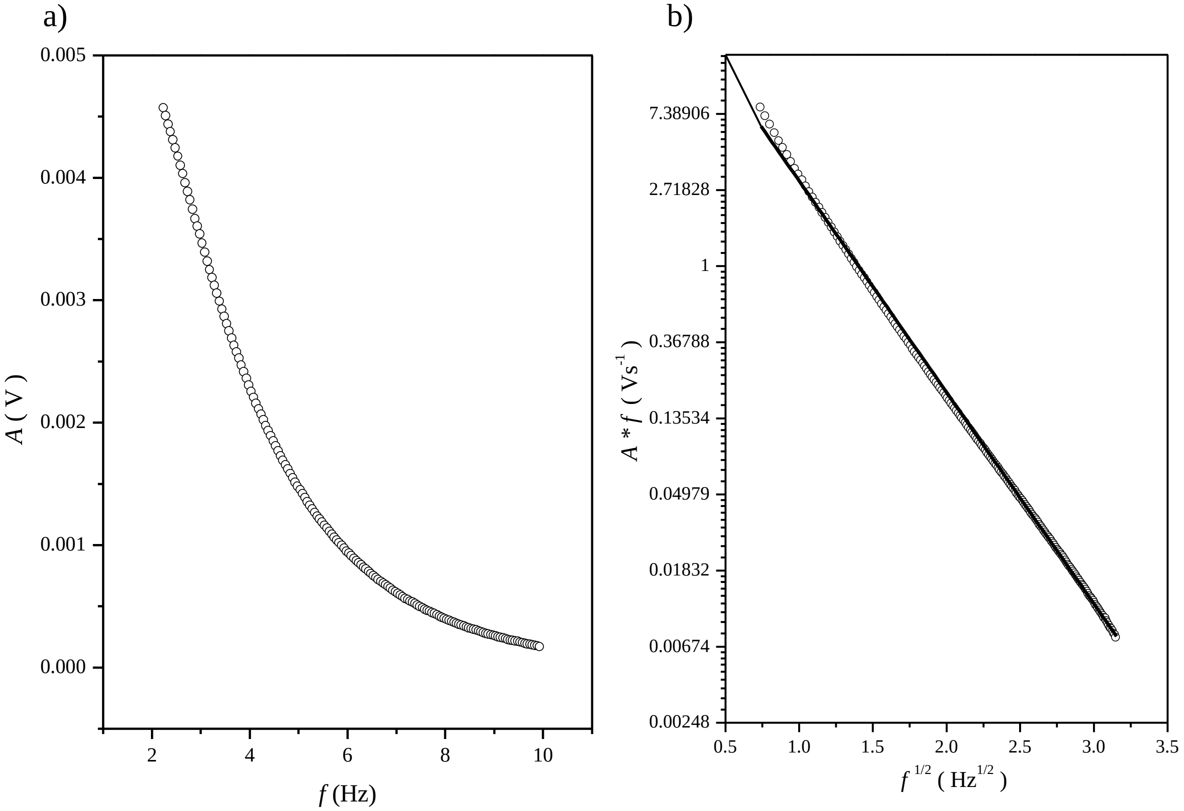

**Fig. 5.3**. a) Typical curve of the PA signal amplitude (*A*) as a function of the modulation frequency ( *f* ) for a sample with 11.8 % of moisture content taken from the middle zone of *Guadua* culm. B) Logarithm of the *A*f* product plotted as a function of the square root of the modulation frequency for the same sample considered in (a). The solid line corresponds to the best least squares linear fit as given by the RG model for an opaque and thermally thick sample (Eq. (1.1)).

The Fig. 5.3. (a) shows a typical curve of the PA signal amplitude (*A*) as a function of the modulation frequency ( *f* ) for a sample taken from the middle zone of a *Guadua* culm with 11.8 % of moisture content. To achieve the optical opacity condition of the RG model the samples were coated with a thin carbon black layer on the surface where light absorption takes place. Similar curves have been recorded for the other samples. In part (b) of the figure we show the logarithm of the *A*f* product as a function of the square root of the modulation frequency, so that according to Eq. (1.1) from chapter 1 the value of α was calculated from the slope of the graph, namely $L\ (\pi/\alpha)^{1/2}$ [14].

Following the above described methodology the thermal diffusivities of all investigated samples were determined. They are plotted in Fig. 5.4 as a function of moisture content for samples collected from the top, middle and bottom regions of the *Guadua* culms. Each represented value is a mean value of 10 independent measurements in different samples prepared under the same conditions. The results of these independent measurements were highly repetitively.

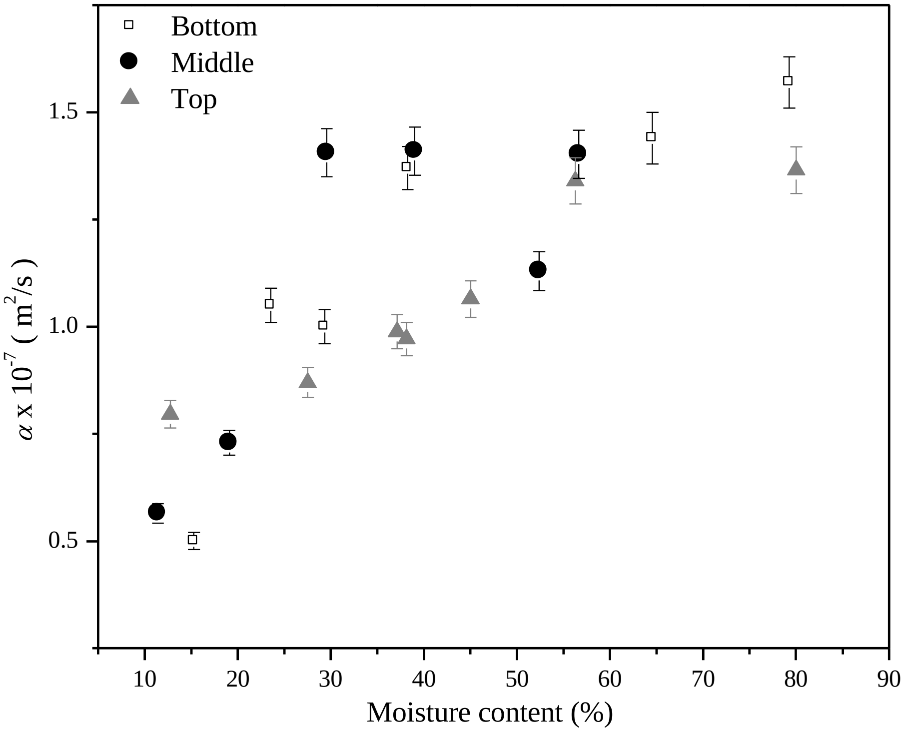

**Fig. 5.4.** Thermal diffusivity values as a function of moisture content for three longitudinal sections of the *Guadua*: bottom, middle, and top.

It can be seen that thermal diffusivity increases as a function of moisture content approaching a saturation value at higher moisture values, being the behavior very similar for samples from the three different regions of the *Guadua* culm, i.e. thermal diffusivity is the same for the three investigated *Guadua* culm zones. This behavior is consistent with previous results obtained for some mechanical properties such as Brinell-hardness of samples taken from the same regions, which do not show appreciable variations between the ranges of the experimental uncertainties [15].

The proportional relationship between thermal diffusivity and moisture content is somewhat an awaited result. If we consider the *Guadua* as a composite material made by cylindrical tubes with solid walls (Fig. 5.5) that for low moisture contents are mainly filled with air and that become filled with a higher than air diffusivity material, as water is, when moisture increases, then the effective medium theories [16] predict an enhancement of the effective thermal diffusivity with the concentration of the second material. According to this we observed how thermal diffusivity enhances from an initial value of $0.05\times10^{-6}$ $m^2/s$ (can be a value for a porous dry wood sample) at the lowest moisture content and approaches a saturation value of $0.15\times10^{-6}$ $m^2/s$ (similar as for some typical woods [6, 17]).

Thermal effusivity measurements were performed using the methodology outlined in Section 5.2.2. A 100 μm silver slab was used as the reference material 1, which was glued using a very thin layer of thermal conductive silver paste to the bamboo’s samples, assuring a good thermal contact. The thicknesses of the bamboo´s samples varied between 200 and 400 μm. The effectiveness of the method used to attach one sample to another has been proved by independent measurements of the thermal effusivity of wood (eucalyptus), stainless steel and glass test samples. For the effective thermal diffusivity measurements the composite samples were located in such a way that they were illuminated on the reference silver sample side, which has been previously carbon blackened for guarantying the optical opacity condition.

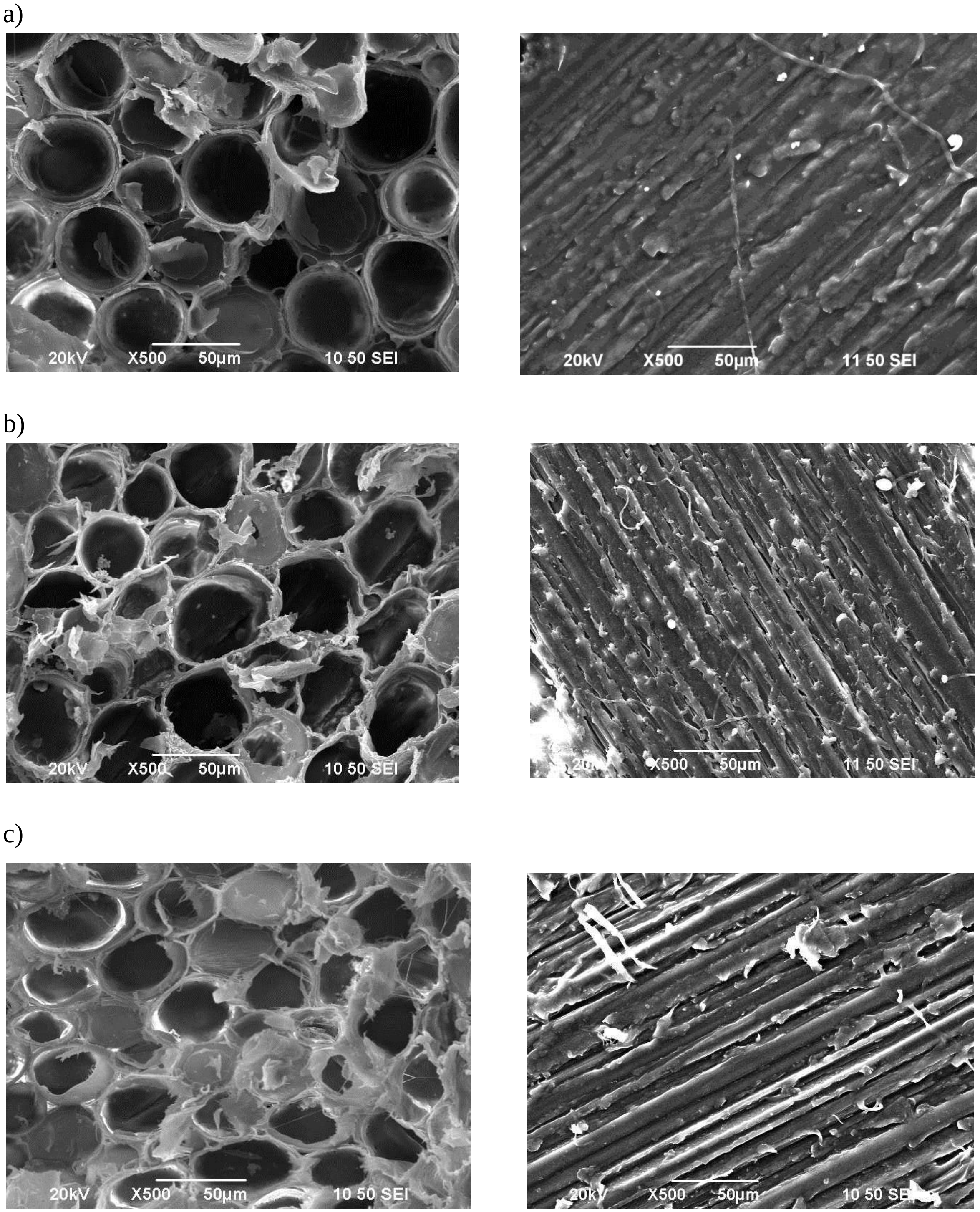

**Fig. 5.5.** SEM micrographs of samples taken from: a) bottom, b) middle and c) top of longitudinal zone in *Guadua* with 11.8% moisture content. The three images showed on the top correspond to a cross-section view while those of the bottom are photographs of a lateral view.

The Fig. 5.6 shows the measured thermal effusivity as a function of the square root of the thermal diffusivity for samples taken from the top culm region of the Guadua for different moisture contents. Following Eq. (5.2) the slope of the curve resulting from the best linear least squares fit of the data is the specific heat capacity. We obtain the value $C$=(0.8±0.1)×$10^{-6}$ J/$m^3$K that is about one order of magnitude smaller than the value of 3×$10^{-6}$ J/$m^3$K predicted for most solids, but, as expected, is in good agreement with that reported for some porous materials such as foams, cork and some woods [18]. For these materials due to their low density there are fewer atoms per unit volume so that $C=\rho c$ becomes low. This is also the case of the bamboos studied here. The principal result is that they have low thermal conductivities and diffusivities, a fact that make them appreciate materials to be used for insulation.

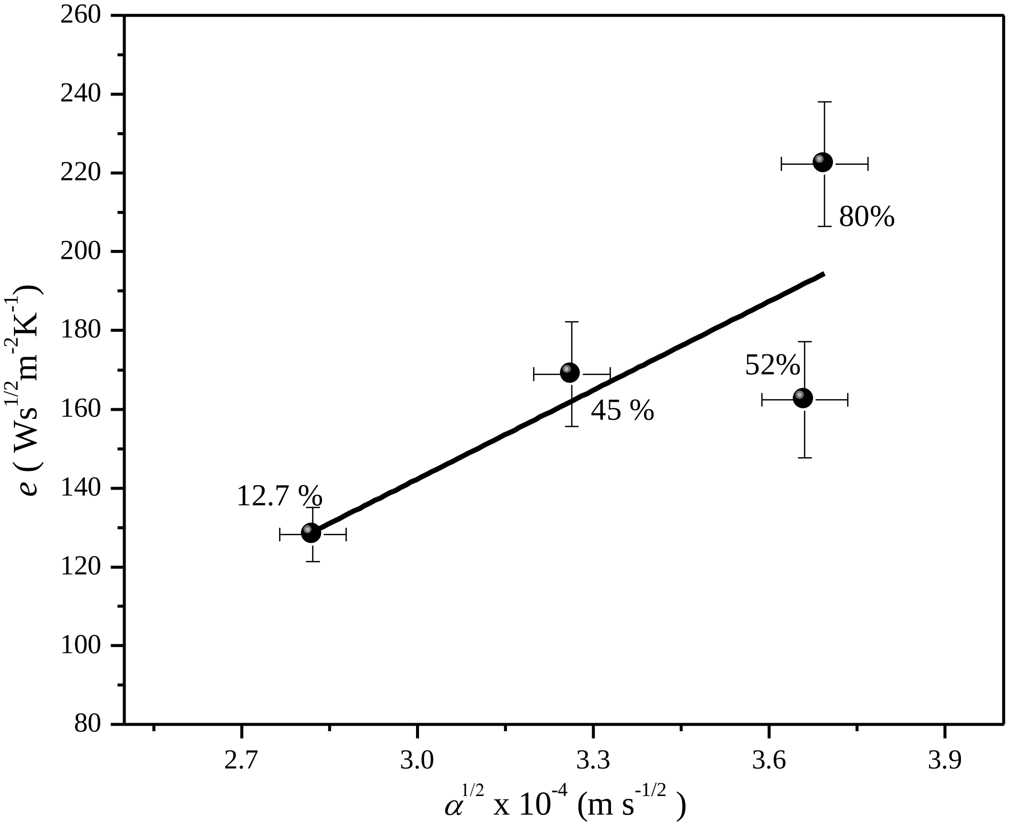

**Fig. 5.6. T**hermal effusivity as a function of the square root of the thermal diffusivity for samples taken from the top culm region of the Guadua for different moisture contents. The solid line is the best least squares linear fit whose slope is equal to the volume specific heat, $\rho C$.

The SEM images of Fig. 5.5 show micrographs of the cross-section and a lateral view of samples from the bottom, middle and top zones. It is possible to see that the vascular bundles diameter is uniformly distributed along *Guadua*. From the micrographs the average diameter of these fibers was estimated as (43.8±1.3) µm, (37.5±1.3) µm and (36.1±1.1) µm for bottom, middle and top zone, respectively. The homogeneity in thermal properties values obtained along *Guadua* culm can be explained considering the size distribution of the vascular bundles, from which we expect that heat propagation takes place through tubes of thin wall with similar composition (the most important elements detected using energy dispersive spectroscopy –EDS JED/2300- were C, O, Si, K, Ca).

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# CHAPTER 6

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## BACTERICIDAL ACTIVITY OF $TiO_2$ FILMS APPLIED TO THE CHARACTERIZATION OF PLANTS GROWTH-PROMOTING BACTERIA

## 6.1. Introduction

Nowadays molecular methods are widely used for studying micro-organisms in the soil, especially those beneficially to plants. As has been mentioned before, these products have positive environmental impact, because when they are applied to plants there are not residuals of chemicals nutrients that generate eutrophication, which cause drastic decreasing in water quality of rivers, lakes and sea. However, the techniques for characterization and identification of micro-organisms are limited by the number of available molecular markers. For that reason, there is a constant search of new methods that complement the found information about microbes and their mutual interaction.

Heterogeneous photocatalysis became an alternative method for air and water purification as a very efficient tool for bacterial disinfecting and for removing organic pollutants in the environment. This catalytic effect has shown a high potential in applications related to gas and liquid phase pollution control processes, and renewable hydrogen production [1, 2]. One of the most common photocatalyst is titanium dioxide ($TiO_2$), a wide band gap, cheap, reusable, nontoxic, photocorrosion resistant, and high oxidant semiconductor. However, the efficient use of sunlight for photocatalysis with this material needs a photoexcitation energy threshold lower than the corresponding to the bulk material (3.2 eV). One way to achieve this is by doping the $TiO_2$ with transition metals, metallic and non-metallic impurities [3].

The photocatalytic efficiency evaluation has been frequently measured by spectrophotometric methods using the methylene blue blanching as a function of time when this is putted in contact with a photocatalyzer radiated with light. However, this technique does not allow the observation of gases generation during the process and includes light dispersion problems.

In this chapter, *in-situ* evaluation of photocatalytic effect of $TiO_2$ films was done using the PA Technique. As has been described in Chapter 1, this effect can be detected as a sound inside a cell that encloses the sample. If in addition, the sample presents photochemical activity, the production of gases contributes to this change. Then, when PA measurements are resolved in time it is possible to study, for instance, photocatalytic activity. The photocatalytic effect of Ag doped $TiO_2$ films and the behavior of bacteria of different species interacting with light have been evaluated using the PA technique, which is relatively cheaper than others. Furthermore, bactericidal photocatalytical effect experiments using these nanostructured semiconductor films on these microorganisms were done using the same technique. The results show that it is possible to use time resolved photoacoustics aided with photocatalysis for identifying bactericidal effects and these species of bacteria.

## 6.2 Experimental

The $TiO_2$ films were grown by the sol-gel technique [4] using titanium tetraisopropoxide as alcoxide precursor and nitride acid as catalytic acid. Silver was incorporated from an $AgNO_3$ dissolution, with concentration changing from 5 to 30 %. In this process nitric acid was used as a catalyzer. The water/alcoxide ratio was fixed in 3 and the films were deposited on glass slide substrates by a dip-coating method. After drying at 210 $^{o}$C for 15 minutes, the films with six impregnation layers were treated in air at temperatures of 600 $^{o}$C and 650 $^{o}$C during one hour. Phase identification of the nanocomposite thin films was conducted with an X-ray diffractometer (XRD) using Cu-$K_{\alpha}$ radiation (D8 Advance Bruker).

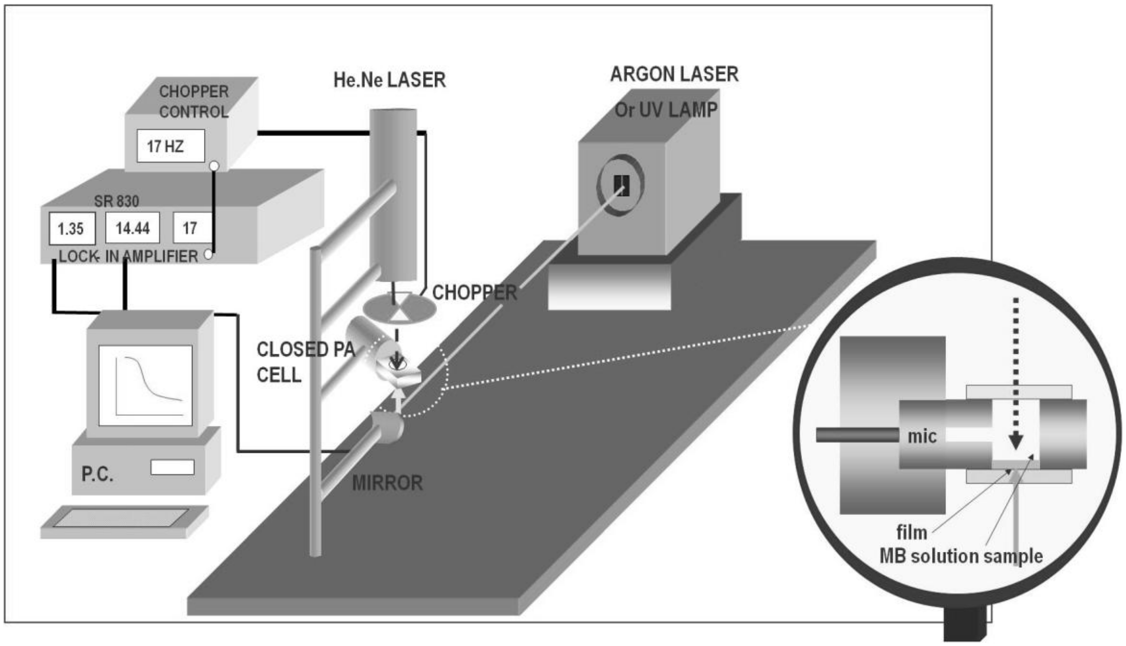

**Fig. 6.1.** PA system arrangement used for photocatalytical evaluation. Modulated light from a He-Ne laser is absorbed by the MB solution. Non-modulated light from an Argón laser or Xe lamp is turned on after 200 seconds to generate a photocatalytic process.

The PA effect was used for analyzing the transformation of methylene blue (MB) solution due to photocatalytical activity and bactericidal effect of $TiO_2$ films using a system like that shown in figure 6.1. The bottom opening of the PA cell is closed by the samples in such a manner that the film, with a drop of the MB solution, faces the cell´s cavity. The top opening is closed with a quartz window through which the mechanically chopped light from a Helium-Neon laser (JDS Uniphase) impinges on the solution-sample system or bacteria-sample system. After 200 s approximately, light from a Xe lamp (ORIEL 66924) irradiated the system from above. A conventional electret microphone located inside the cell detected the PA signal, which is fed to a SR850 lock-in amplifier synchronized at the chopped light frequency. The PA signal is produced by the photothermal effect due to the chopped light absorption and by the photocatalytical effect when the Argon laser beam or the Xe lamp is turned on.

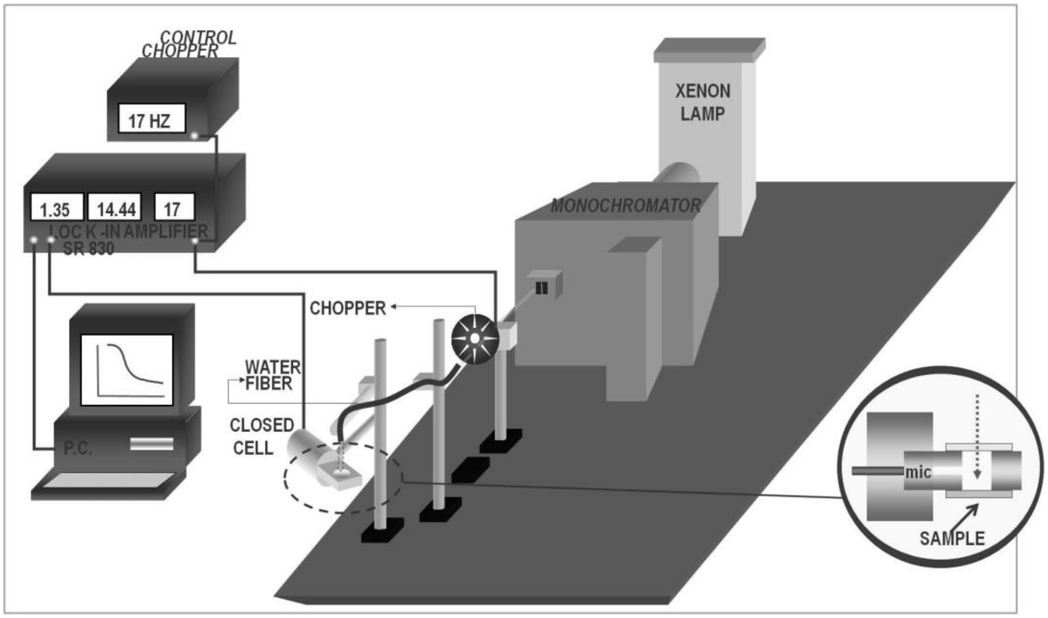

**Fig. 6.2.** PAS system with a closed PA cell. The thin film grown on glass slide was used as cover of the cell cavity.

PA spectra of the films were measured using a system showed schematically in figure 6.2. The sample is cyclically heated by chopped light from the Xenon arc lamp (ORIEL 66924) using 700 W power. A monochromator (ORIEL Cornerstone 130 1/8 m) was used for selecting the light wavelength. The monochromatic modulated light beam is focused on the sample using a liquid light guide. The light energy absorbed by the sample excites electrons to higher levels; the non-radiative de-excitation processes heats periodically the sample and thus its surrounding gas within the closed PA cell. The oscillatory motion of this gas layer produces an acoustic signal detected in the cell by the electret microphone, and the intensity of this signal has a direct correspondence with the amount of light energy absorbed by the sample [5]. A lock-in amplifier was also used for reading and amplifying the signal from the transducer.

### 6.3 Results and discussion

Non-doped $TiO_2$ films were previously growth to find optimum parameters in order to obtain better photocatalytic activity. The number of impregnated layers (3-11) and the sintering temperature (600 and 650 $^0$C) were changed during the growing. In the whole set of films the same crystalline structure of anatase phase was found and the grain size and band gap were measured as 20 nm and 3.3 eV, respectively. However, the photocatalytic activity have changed with sintering temperature and number of impregnations. This is probably because the porosity of the films was modified with these parameters.

The X-ray diffractograms showed that the crystalline structure of the $TiO_2$ films does not depends of the used growth parameters. A peak around $2\theta=25^0$ in these patterns revealed preference for anatase phase in agreement with reports for $TiO_2$ films grown by this technique [5]. From the peak full width at half maximum (FWHM) in these diffractograms, using Scherrer´s equation the average nanometer grain size was estimated [6].

The forbidden band gap energy of the films was calculated from UV-Vis absorption spectrum assuming indirect allowed transitions close to the fundamental absorption, using the following relationship:

$$\alpha h\upsilon \sim A_i (h\upsilon - E_g)^2 \tag{6.1}$$

Here $\alpha$ is the absorption coefficient, $h\upsilon$ is the photon energy, $A_i$ is a photon energy independent parameter for respective transitions, and $E_g$ is the optical bandgap energy [7]. The values obtained from the fitting to the above equation are shown in Table 6. 1. One of them corresponds to the anatase phase, reported as 3.2 eV [8], while the other one is probably due to shifting by impurities.

The photo-blanching of MB with photocatalysis was measured using visible spectrophotometry. The concentration of MB solution after the photocatalytic process with the films of titanium dioxide, the grain size, and the forbidden gap energy of these films are shown in Table 6.1.

**Table 6.1.** Sintering temperature ($T_s$), grain size (L), forbidden gap energy ($E_g$) and concentration of MB solution in contact to $TiO_2$ film after 5 hours of UV irradiation. For all set of the films water/alcoxide ratio was 3.

| $T_s$ [ºC] | Number of impregnations | L ±1.6 [nm] | $E_g$ ±0.05 (eV) | Concentration of MB [µM] |
|---|---|---|---|---|
| 600 | 4 | 18.0 | 3.40 | 11,3 |
| | 6 | 21.0 | 3.20 | 12,6 |
| | 7 | 21.0 | 3.20 | 2,1 |
| | 8 | 21.0 | 3.30 | 0,6 |
| | 9 | 20.0 | 3.40 | 0,9 |
| | 10 | 19.0 | 3.30 | 8,4 |
| | 11 | 17.0 | 3.40 | 8,5 |
| 650 | 4 | 22.0 | 3.30 | 13,4 |
| | 6 | 20.0 | 3.30 | 4,9 |
| | 7 | 19.0 | 3.30 | 7,8 |
| | 8 | 19.0 | 3.20 | 1.1 |
| | 9 | 19.0 | 3.30 | 14,4 |
| | 10 | 19.0 | 3.40 | 18,6 |
| | 11 | 17.0 | 3.30 | 20,3 |

In Fig. 6.3 is possible to see that minimum values of MB concentration after photocatalytic process were obtained using the $TiO_2$ films with 8 and 9 layers. Although the behavior of this concentration as a function of layers number is the same for both sintering temperatures, 600 $^0$C was chosen as a better parameter because the minimum value of MB concentration (0.6 µM) was obtained using it. Also a layer number of six was chosen as a good value for growing of $TiO_2$ doped-films.

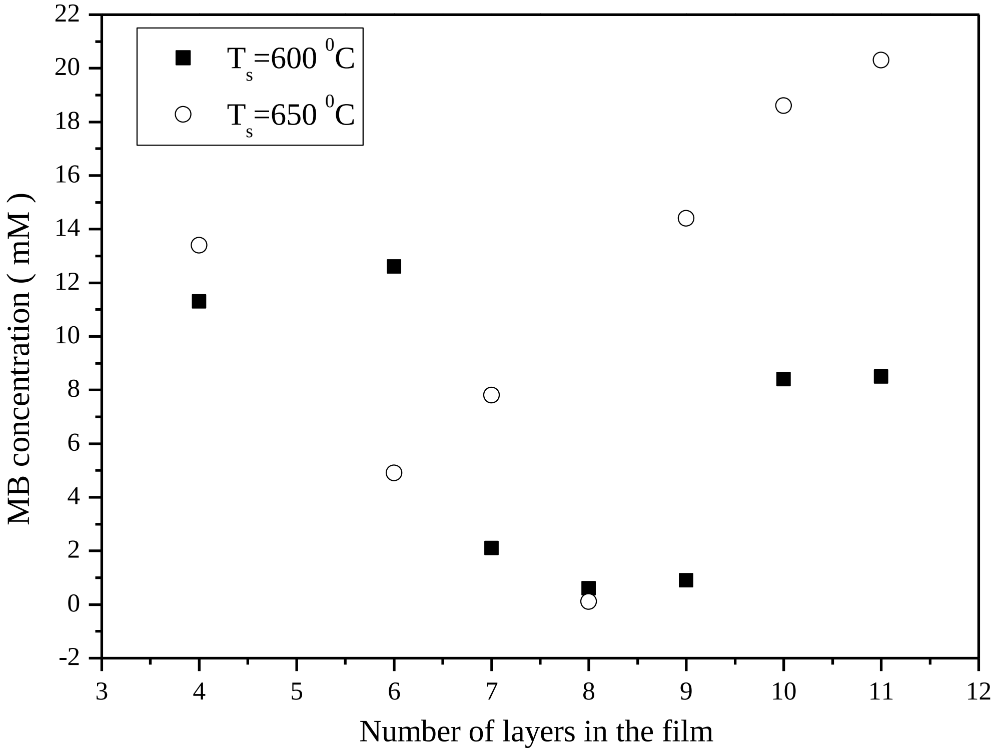

**Fig. 6.3**. Concentration of MB solution after photocatalytic process in function of the number of layers in the $TiO_2$ films. Squares and circles correspond to 600 $^0$C and 650 $^0$C sintering temperatures respectively.

The X-ray diffractograms in figure 6.4 showed that the crystalline structure of $TiO_2$ doped-films neither depends of the used parameters. Also the same peak around $2\theta=25^0$ in these patterns revealed preference for anatase phase. From the FWHM in these diffractograms, the average nanometer grain size was estimated to be in the range 17-30 nm using Scherrer´s equation. So, it is possible to say that inclusion of Ag in the films of $TiO_2$ can increase the size of nanocrystals in the film.

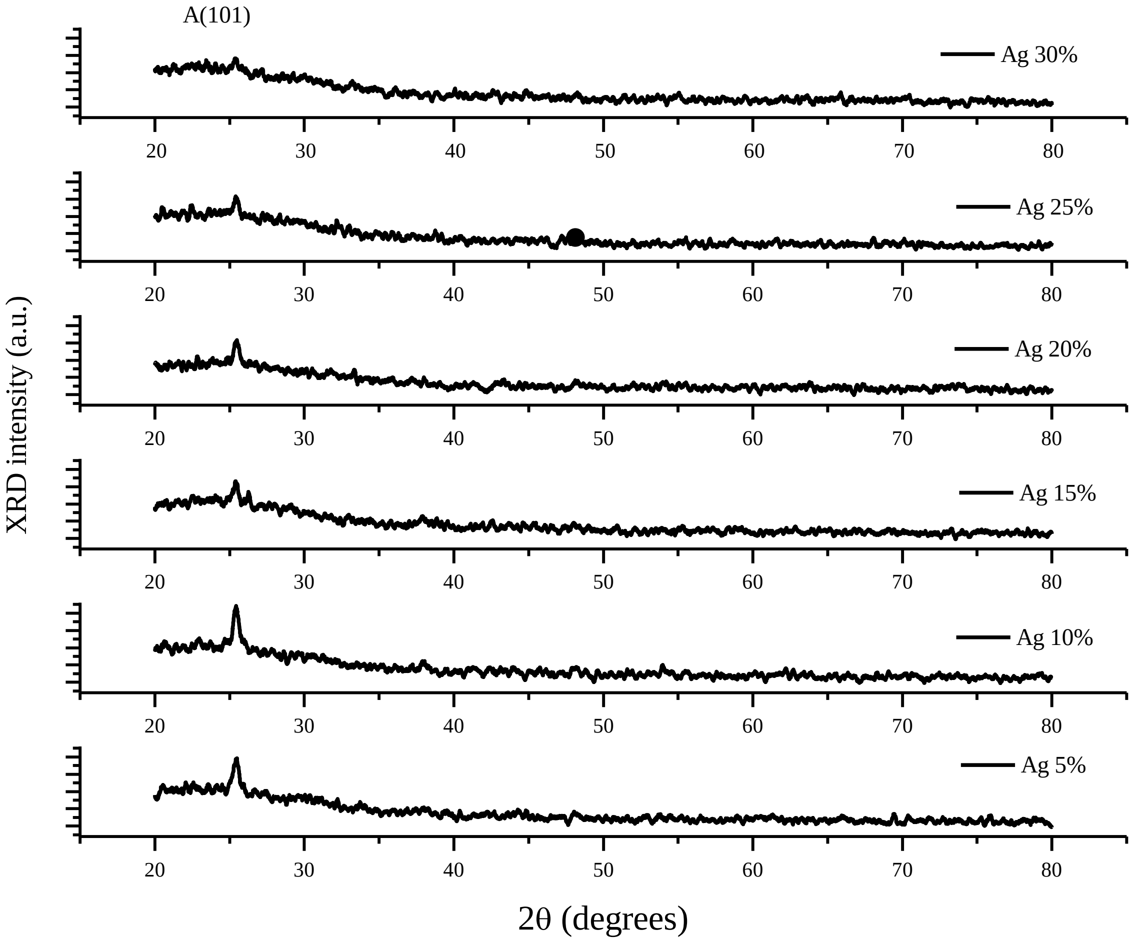

**Fig. 6.4**. Difractograms for all $TiO_2$ grown films show a peak related to (101) anatase phase. Samples were called according to $AgNO_3$ dissolution concentration in growth process which was changing from 5 to 30 %.

Incorporation of Ag in $TiO_2$ films was corroborated by energy dispersive spectroscopy (EDS). Fig. 6.5 shows the spectra for Ag25 and Ag30 samples discounting Si from the glass substrate; other elements such as Mg (MgO), Al ($Al_2O_3$), Ca (CaO) and Na ($Na_2O$) in the spectra also correspond to glass. The weigh percentage was 0.84 and 1.04 in Ag25 and Ag30 respectively, subtracting Si. This indicates a proportional relation among these values and the concentration of $AgNO_3$ dissolution used in sol-gel preparation.

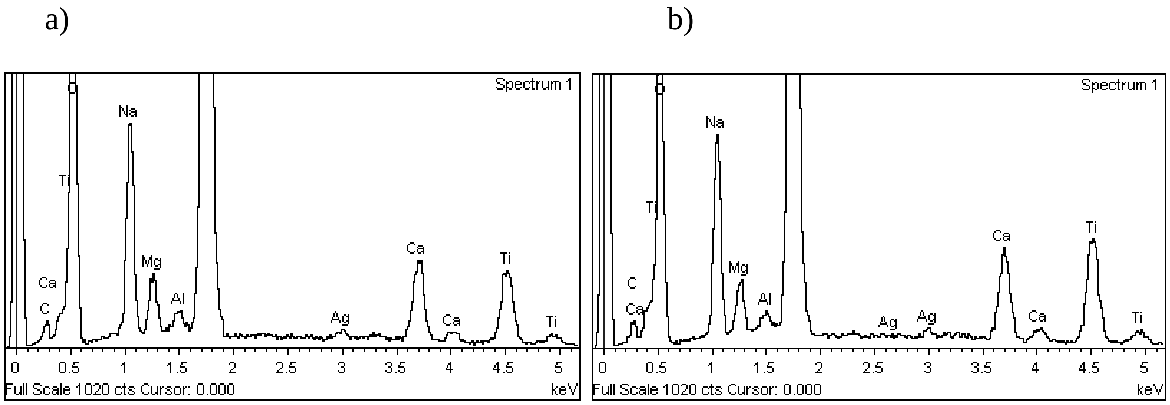

**Fig. 6.5.** EDS spectra for a) Ag25 and b) Ag30 samples.

The forbidden band gap energy of the films was also calculated from absorption spectrum (Fig. 6.6) assuming indirect allowed transitions; the calculated values are shown in Table 6.2. It is possible to see that the addition of $AgNO_3$ caused a shifting of the band gap value in a range between 2 and 2.3 eV according to values obtained from the fitting to the equation (6.1).

**Table 6.2.** Grain size (S) and band gap energy ($E_g$) estimated for $TiO_2$ films using Scherrer equation and indirect transitions model (equation (6.1)), respectively.

| Sample | S [nm] | $E_g$ [eV] |
|---|---|---|
| Ag 5% | 18 | 2,3 |
| Ag 10% | 23 | 2,1 |
| Ag 15% | 24 | 2,0 |
| Ag 20% | 25 | 2,0 |
| Ag 25% | 29 | 2.0 |
| Ag 30% | 30 | 2.0 |

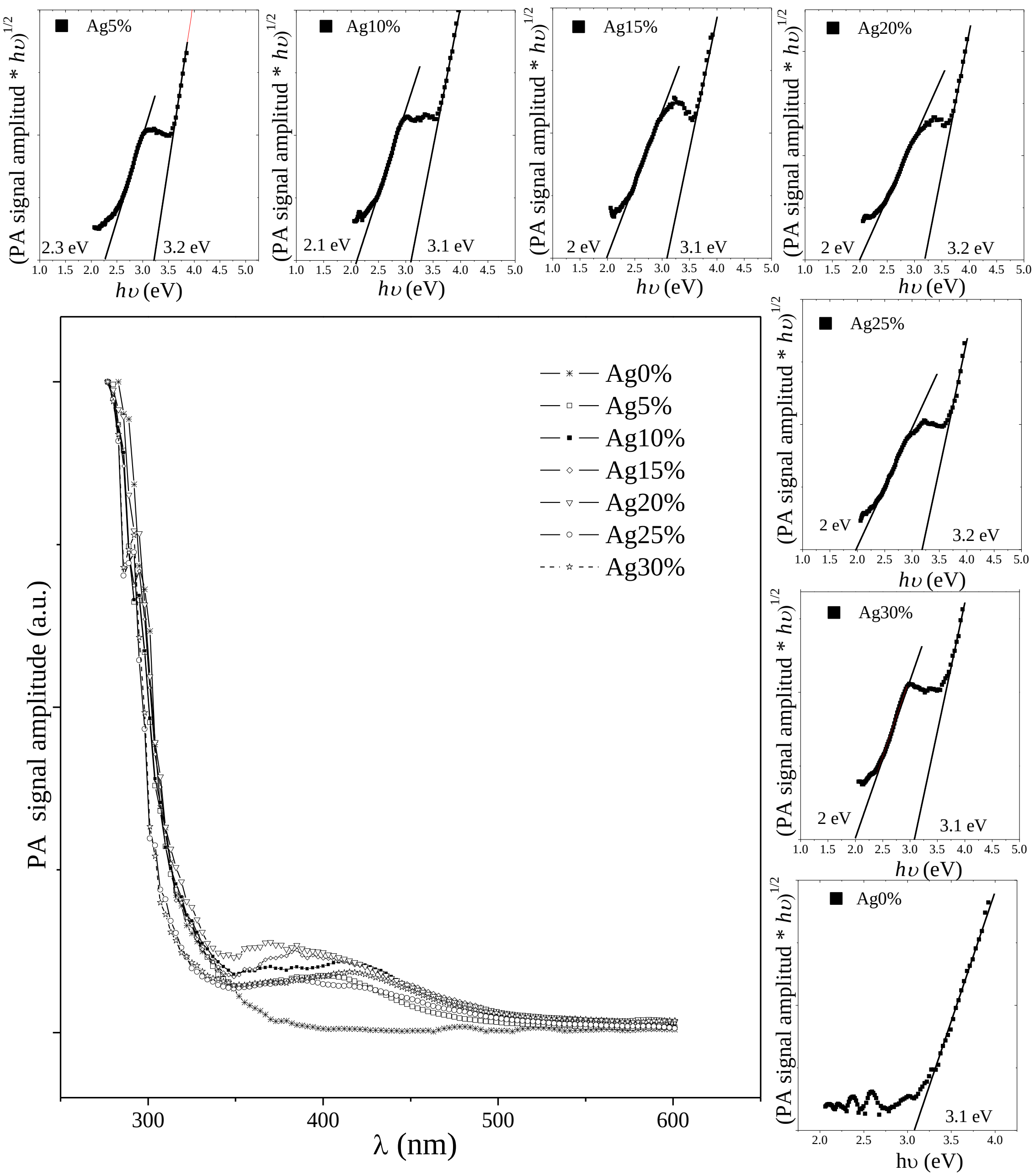

**Fig. 6.6.** Photoacoustic spectra of the films. The insets show the graphs of (PA signal amplitude $*h\upsilon)^{1/2}$ vs. $h\upsilon$. The values of the corresponding optical band gap were estimated by extrapolation of an apparently narrow linear region according to equation (6.1).

Photocatalytic activity with visible light of these $TiO_2$-doped films was evaluated firstly via conventional visible spectrophotometry. The obtained spectra are shown in Fig. 6.7. The initial concentration of the MB solution was 60 µM, which can be decrease 38% after photo-blanching using Ag15 sample and white light by 20 hours.

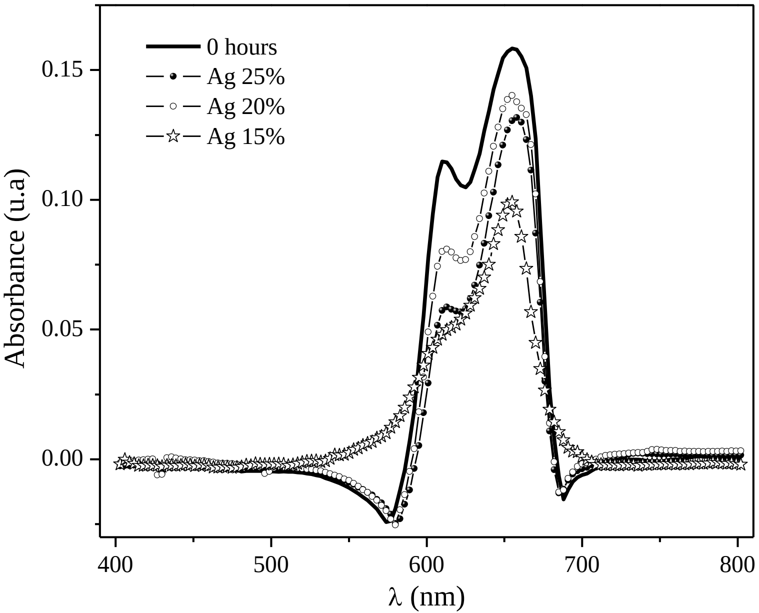

**Fig. 6.7.** Spectra of MB solution after photo-bleaching. This photocatalytic activity was measured with spectrophotometer. White light from a Xenon lamp was used for the photo-activation during 20 hours.

Ag25% sample was used for testing the potential of the PA technique for sensing the photochemical activity with white light. As expected, the time-resolved PA signal curves indicated that the photocatalytic activity, measured *in situ* through the methylene blue solution, changes due to degraded molecules, water cleavage and possible generation of other gases.

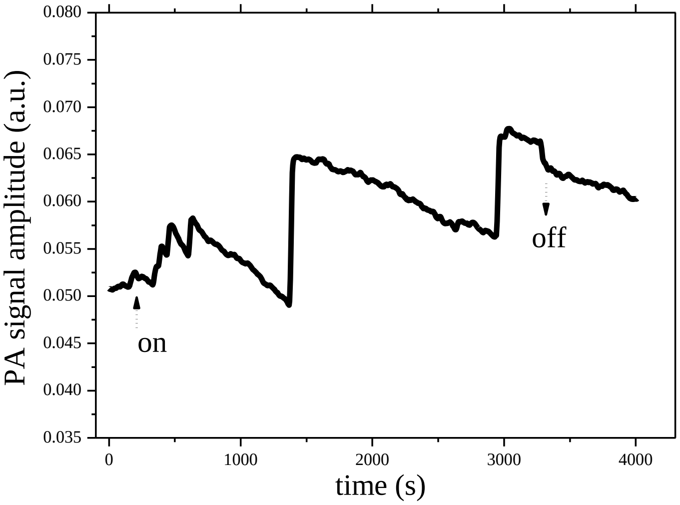

**Fig. 6.8.** PA amplitude as a function of time of a MB solution during photo-bleaching. In this case, Ag25% sample and white light from a Xenon lamp were used for the photo-activation during 4000 s.

The figure 6.8 shows the PA signal as a function of time when chopped light is absorbed by the MB solution and non-modulated light from a Xe lamp is irradiated on the MB solution-film system, after the first 200 s. Photocatalytic effect can be observed from this curve. It is also possible to see that the signal intensity increases with the time, so that real blanching didn´t takes place in this period, but other degradation process with gas generation. In this case, the PA signal corresponding to the photochemical effect is an evidence of the photocatalytic activity using white light. With this information, the bactericidal effect of the films was tested in the same manner.

In the Fig. 6.9, the PA signal amplitude as a function of the time for a bactericidal experiment using the Ag-doped $TiO_2$, Ag15% is shown. The curves correspond to the PA response of *Burkholderia unamae* (Strain TATl-371) to white light and to the PA response including the photobaric effect due to the photocatalytic activity of $TiO_2$ film on the same bacteria.

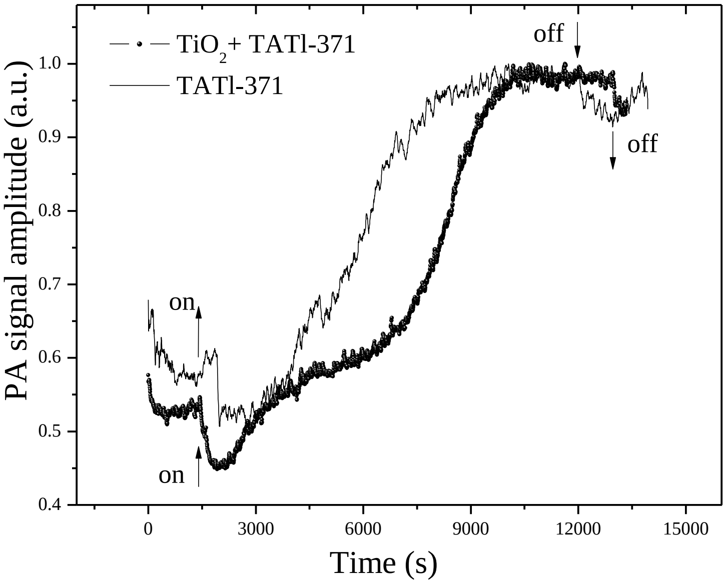

**Fig. 6.9.** PA signal amplitude as a function of the time. The solid line corresponds to the PA response of *Burkholderia unamae* (Strain TATl-371) and the dotted curve corresponds to the PA response including the photobaric effect due to the photocatalytic activity of $TiO_2$ film on this same bacteria. The up and down arrows indicate the time instants at which a non-modulated white light Xe-Lamp, used to induce the photocatalytic process, is turned on and off respectively.

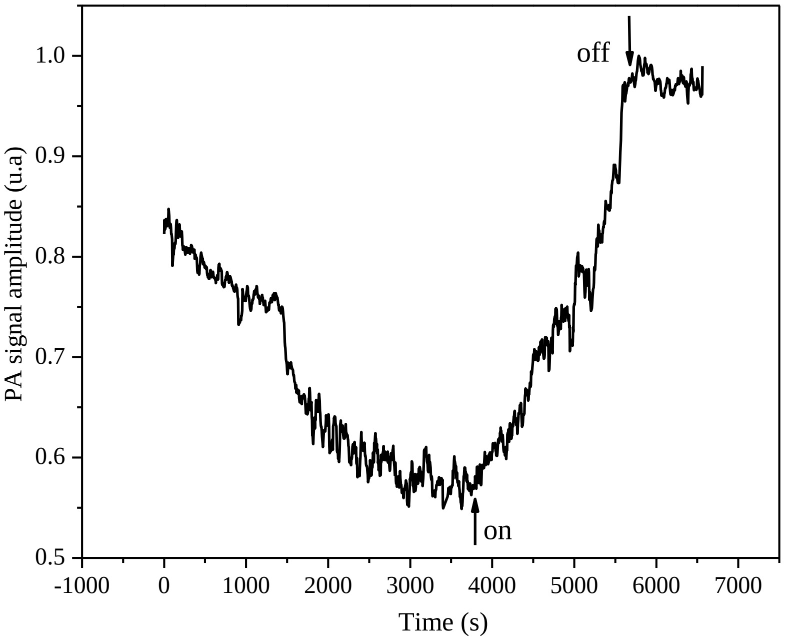

**Fig. 6.10.** PA signal amplitude as a function of the time. The curve corresponds to the PA response of *Burkholderia unamae* (Strain Strain MTI 641) including the photobaric effect due to the bactericidal activity of $TiO_2$ film on this same bacterium. The up and down arrows indicate the time instants at which a non-modulated white light Xe-Lamp, used to induce the photocatalytic process, is turned on and off respectively.

The experiment was repeated using other strain of the same species, *Burkholderia unamae*, the Strain MTI 641. The PA signal amplitude as a function of photocatalysis time using the same sample Ag15% was monitored in the same way as in Fig. 6.10. The behaviour of this curve is different to that shown in Fig. 6.9 corresponding to Strain TATl-371. This can be taken as an evidence of the potential application of this method for characterization of plant growth-promoting bacteria.

## 6.4 References

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# CONCLUSIONS

In this thesis three modes of operation in PA technique, namely resolved in wavelength, in modulation frequency and in measurement time, were used for the study of environmental problems related to "clean agriculture". The results proved the potentials of the PA technique for contributing to the development of environmental friendly practices. Advantages of photothermal methods such as the cheap cost, the wide dynamic range, the possibility they offers to perform optical measurements in highly opaque samples, and in those in the form of powers, gels, etc. were well exploited in this work. The lesser influence from light scattering when compared with optical techniques, the non-necessity of electrical contacts for transport properties measurements, the monitoring *in situ* of photochemical processes and the straightforward mathematical formalism behind them, were well used too.

Agricultural cultivation practices should be environmental friendly and clean for avoiding damages to health of producers, consumers and, of course, of the earth. Educative programs, cheapest and efficient certification process, biotechnology application, fair trade, and non-monopoly of this market can serve for decreasing the price of eco-materials and organic products. In this work we showed some efforts for the physical characterization of coffee, rice, guadua and the effects of plants growth stimulating bacteria using non-conventional techniques that can contribute to some of these strategies. The here presented techniques are based basically in the photothermal effect, in which the thermal state of a sample is disturbed in a non-invasive and non-destructive way so that the induced changes carry out information about optical and thermal properties of the sample and about the photochemical processes occurring as a result of the absorption of electromagnetic energy.

Four specific problems of cleaner agriculture were explored in this work with the use of the PA technique. Firstly, the search for criteria for discrimination of organic products was made through the particular case of coffee and rice. Secondly, the evaluation of the effect of growing promoting bacteria on coffee and maize plants was performed. Thirdly, thermal characterization of *Guadua angustifolia*, also known as the “bamboo of the Americas”, has been performe. This is an ecological material that avoid the exclusive use of classical tree woods in the building industry. Finally, the photocatalytic activity of titanium dioxide on growing promoting bacteria was studied using the same technique, looking for a cheap method for characterizing these strains.

It has been showed how the determination of thermophysical parameters, such as thermal diffusivity, as well as spectroscopic measurements in roasted and ground samples of organic and conventional coffee, can serve as certification criteria because they depend on pigmentation, chemical composition, structure and distribution of components in the bean, which can be probably affected by the chemicals that can be inoculated in the grain, after or before the harvest. Thus, measurement of thermal diffusivity and MIR-visible spectra of organic and conventional coffee can open possibilities to possible discrimination criteria.

The analysis of morphoanatomical characteristics of coffee beans by microphotography showed that according to the type of crop techniques, organic or conventional, the distribution of the nutrients and pigments change. These results are in agreement with pigmentation and thermal diffusivity characteristics obtained from PA measurements resolved in wavelength and modulation frequency. Cellular structure of organic coffee is more homogenous and it presents less quantity of damage cells. These histological characteristics can be studied in depth for distinguishing between each kind of coffee.

Particularly, infrared spectroscopy study of roasted and ground coffee from *Coffea Arabica* grown by organic and conventional methods on four geographical altitudes was done using the PA technique. Results allowed collecting information about coffee powder spectral behavior for establishing some distinctiveness of coffee chemical composition. It can be proved that spectral curves of organic coffee are more affected by geographical altitude than conventional coffee. Whole solar exposition and agrochemicals used in conventional cultivation techniques are a possible explanation to this behavior. These characteristics were identified eliminating light dispersion and without special preparation of the samples.

Photosynthetic activity was monitored using also the PA technique in coffee plants, which were treated in isolated manner with synthetic fertilizer and biofertilizer. Results showed that any treatment causes effects over photosynthesis process and stomata response. In particular, it has been found that the application of biofertilizers has a large effect on oxygen evolution ratio without strong alteration of openness of light exposed stomas respecting to those of control group plants. So, this methodology can be used for evaluating bacterial inoculants, which have enormous potential importance for diminishing leaching of residues of fertilizing and the use of pesticides.

The differences in microphotographs and thermal diffusivity values of the rice according to type of growing treatment demonstrated the homogenous distribution of the nutrients and pigments in the organic rice. Thermal diffusivity was approximately 40% lower for the organic samples.

The apparent presence of starch was almost two times higher in organic rice that in conventional sample. These results can be explained by different internal distribution of components evidenced in micrographs.

It was possible to identify using PAS certain characteristics of the rice grown with organic techniques, when the presence of rice pigments such as capsaicin, β-carotene, lutein, violaxanthin and neoxanthin are considered. From the criteria of the first derivative and the analysis of variance, important differences in the form of PA spectra corresponding to the cultivated samples with both types of treatment were determined.

According to spectroscopy results, both conventional and organic rice have similar pigments content. However, the spectra corresponding to husk show different pigments distribution in each case. Capsaicin and capsorubin were specially identified in husk of organic rice around 360 and 411 nm, respectively; which characterizes the origin of the sample.

These results guarantee the use of PA technique as a tool that would allow discrimination of rice growing by organic practices. On the other hand, the study of the physical properties of the rice grain and the rice husk opens the way to future applications of this raw material in transformation processes.

A photosynthetic activity monitoring for maize plants with two bacterial inoculants treatments was done using the PA technique. The results show that both treatments cause effects on the photosynthesis process. In particular, we observed the same effect of the two bacteria on the oxygen diffusion coefficient, which becomes approximately 20% lower than that of the control group plants. The observed fact that the OER values are different in both cases can be probably an indication of non-photosynthetic factors influence on the measurements, such as the leaf internal anatomy, which is evidenced by the thermal diffusivity behavior.

These results establish changes in maize photosynthetic activity due to plant-microbe interaction. The experimental conditions guarantied the isolation of other variables that are considered in conventional field studies.

The photoacoustic technique used in this work is a relatively novel, well argued, and cheap method that allowed us to perform in situ and in vivo measurements. We believe that the results presented here can contribute to a better understanding of bacterial inoculants effects on plants for “biofertilizers” development.

We also demonstrate that the photoacoustic technique allows measurement in a cheap, efficient and non-destructive (in the sense that the technique does not require the use of electrical contacts, as in some more conventional methods) way of the principal properties involved in heat transfer of *Guadua*. Thermal diffusivity was measured using this technique for samples from different *Guadua* sections: bottom, middle, and top. Results show that thermal diffusivity increases with moisture content, but it not shows important changes along *Guadua* culm, a fact that has been explained on the basis of the homogeneity in morphological characteristics evidenced by SEM microphotographs taken from cross and longitudinal section of the bamboos. These images showed that the area occupied by the vascular bundles (parenchyma and other fibers in the stem) remains approximately invariable along *Guadua* culm.

The in-situ analysis using the PA technique of the photocatalytical action of $TiO_2$ films with different Ag contents, showed high efficiency for monitoring this activity. Thin films PA spectra show than inclusion of Ag in the growth process of these films produces a shift of the forbidden band gap energy values, which enhances the films photoactivity with white light. The *in situ* monitoring, with the same PA configuration, of the interactions of $TiO_2$ films with plant growing promoting bacteria, shows particular characteristics that could serve for identifying these species.

The research results presented in this work do not pretend to establish strategies for resolving environmental problems caused by agricultural activity. Although the damages can be reverted an enormous compromise must be acquired and this is a work of disproportional dimensions. However, we hope that the contributions reviewed here show the possibilities that photothermal methods can offer in order to diminishing the commercial cost of organic production, which has healthy characteristics that favors not only human but environment.

In summary, the following goals were completed in this work:

1- Information was found using the PA technique, about characteristics of organic coffee for defining possible certification criteria.

2- The PA technique was used for monitoring the quality of coffee.

3- The photosynthetic activity of coffee plants was monitored using the PA technique as a way to study the effect of bacterial inoculants application.

4- Information about characteristics of organic rice was found using the PA technique that can help to define possible criteria for certification.

5- The time resolved PA technique was used for measuring the photobaric process in maize plants from seeds inoculated with two beneficial bacteria: *Azospirillum brasilense* and *Burkholderia unamae*.

6- The thermal diffusivity was measured as a function of the moisture content obtained during the drying process in samples of *Guadua angustifolia* taking from the bottom, middle and top culm regions of the plants.

7- The photocatalytic activity of titanium dioxide films on a solution of methylene blue using the PA technique was tested.

8- The bactericidal effect of titanium dioxide films on beneficial bacteria of plants was studied using the PA technique.

# FINAL COMMENTS AND FURTHER WORK

This study was principally focused to applications of the PA technique for contributing to cheaper protocols than the conventional used criteria (based mainly on “eye” inspection) for certification of organic coffee and rice, for the characterization of eco-materials such as Guadua, for the evaluation of biotechnological products as biofertilizers for coffee and maize plants and for the study of the photocatalyst effect of titanium dioxide for characterizing plant growth-promoting bacteria.

Although the main objectives of this Thesis have been well fulfilled, e.g. by demonstrating that the PA technique can be a useful tool for the monitoring and evaluation of processes and phenomena that can contribute to the strengthening of “clean” agricultural practices, as in any scientific work there are some questions that require further attention and investigation.

Some limitations have been found when looking for evidences of special characteristics of agricultural organic products or effects of specific treatments allowing the establishment of certification procedures. Firstly, the complexity of the materials of biological origin leads to non-complete control of involved variables so that the final information about these materials is only a statistical approximation. For instance, although the samples of coffee seeds were collected from neighbor crops it is possible that some factors such as local fauna or micro-fauna change the conditions of growing of the plant. However, for practical applications the studies must be performed under conditions so much closed as possible to those encounter in the real agricultural praxis. Secondly, although the PA technique is versatile and the optical microscopy is forceful, the use of other characterization techniques such as confocal microscopy, transmission electron microscopy (TEM), Raman spectroscopy, differential scanning calorimetry (DSC), etc., is needed for validate some results, but the expensive cost of these methods can result a disadvantage for the practical application. Thirdly, for accurate results the measurements of photosynthetic activity using photoacoustics must be done daily during a short period of time, for which an adequate multidisciplinary team has to be employed for simultaneously measuring important physiological variables in treated plants that can have influence on photosynthesis. Thus, a mobile system for measurements *in situ* at the green-house should be of great importance for enhancing the amount of data with more plants and treatments. Such a system must be constructed using compact modulated light sources of high intensity and with a wide range of available wavelengths, such as continuous and pulsed semiconductor lasers or LEDs, as well as the development of portable equipments for the detection of signals with low signal to noise ratio, that can be introduced in a microprocessor controllable embedded system or controlled using a portable computer.

On the other hand, although in this work enough information was found about some organic products such as coffee and rice, the standardization of methods and criteria must be done in order to apply these results to other foods and to propose a formal certification protocol that could be adopted by a certifier company.

Finally, the use of the $TiO_2$-photocatalysis aided PA technique could be an efficient and cheap method for identifying and characterizing beneficial bacteria to be used for fabricating biofertilizers with local specifications. This can increase the efficiency of these biotechnological products. On the other hand, *in situ* monitoring of photocatalysis opens a way for best understanding of bactericidal effect and for research on photoactive materials with visible light for environmental applications.

# ACADEMIC PRODUCTS

## Published and accepted papers

1. F. Gordillo-Delgado, K. Villa-Gómez and E. Marín. Shifting to the red the absorption edge in $TiO_2$ films: a photoacoustic study. Revista Superficies y Vacío, 24(1) 20-23, 2011. Indexed by CONACyT, Latindex, Redalyc, Directory of open access journals and SciFinder.
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8. F. Gordillo-Delgado, D. M. Cortés-Hernández, C. Mejia-Morales. Comportamiento de los parámetros termofísicos de la *Guadua angustifolia* - Kunth medidos con la técnica fotoacústica. Revista colombiana de física. Revista colombiana de física. Indexed by publindex, EBSCO, classified B by COLCIENCIAS. (Accepted for publication)
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## Chapters in books

1. F. Gordillo-Delgado, E. Marín. "Special characteristics of organic coffee obtained via phothermal techniques" accepted for publication in the edited collection, entitled "Coffee Consumption and Health". Nova Science Publishers, Inc. Hauppauge NY, United States of America. Editor Bernard Goodman. (Accepted for publication)

## Submitted papers

1. F. Gordillo-Delgado, D. M. Cortés-Hernández, C. Mejía-Morales, H. Ariza-Calderón, A. F. Bedoya. Dureza Brinell y parámetros termofísicos de la *Guadua angustifolia* – Kunth. Revista materia. Indexed by SCielo.
2. F. Gordillo-Delgado, C. Mejía-Morales, F. Zarate-Rincón. Analysis of photosynthesis activity and morfo-anatomicals characteristics of "*Coffea arabica*" treated with microbiologycal products. Revista Superficies y Vacío. Indexed by CONACyT, Latindex, Redalyc, Directory of open access journals and SciFinder.
3. F. Gordillo-Delgado, D. M. Cortés-Hernández, C. Mejía-Morales, A. J. García-Salcedo, and E. Marín. Discrimination of "organic" coffee via FTIR-photoacoustic spectroscopy. Journal of the Science of Food and Agriculture.

## Papers under progress

1. F. Gordillo-Delgado, E. Marín, C.M. Mejía-Morales. Study of the pigments in Colombian powdered coffee using photoacoustic spectroscopy.

## Presentations in congresses

### International

1. F. Gordillo-Delgado, A. J. García-Salcedo, C. Mejía-Morales. Identification of adulterants in roasted and ground coffee using FTIR-PAS. Segundo Simposio Internacional y Tercero Nacional agroalimentario. Del 9 al 12 de Agosto de 2011. Montería-Córdoba (Colombia).

2. F. Gordillo-Delgado, E. Marín, J.A. Calderón-Arenas. Photoacoustic Response from Soil Bacteria. XIX International Materials Research Congress del 15 al 19 de Agosto de 2010. Cancún (México). Sociedad Mexicana de Materiales, A.C.

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**National**

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