

Evaluation of Wheat and Maize Seeds by Photoacoustic Microscopy

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Abstract In this study photoacoustic microscopy (PAM) was used to evaluate the deterioration of wheat and maize seeds. The analyzed seeds were obtained from different productive cycles. In the PAM experimental setup a modulated focused laser beam impinges on the sample. Due to the periodic heating it is possible to obtain a photoacoustic signal (PA) by using a microphone sensor; from this sensor an electrical signal is obtained due to the temperature variation in the sample. Since there are local differences in seed structure, the optical absorption of the laser beam and thermal properties are inhomogeneous, which produces a different PA signal in each point of the sample. By taking the set of the obtained PA signals as a function of their positions, it is possible to obtain a PA image. The image resolution was 50 μm for

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each seed. Therefore, the main objective of the present study was to obtain PA images of wheat and maize seeds, from different productive cycles (i.e., from different annual cycles). The analysis has shown that PAM is an alternative technique to evaluate seed deterioration. Also, the optical absorption spectra of these seeds were obtained by PA spectroscopy.

Keywords Photoacoustic microscopy · Photoacoustic spectroscopy · Seed deterioration · *Triticum aestivum* L. · *Zea mays* L.

1 Introduction

Photothermal microscopy (PTM) can be used with great advantage in biological studies since this technique is capable to obtain thermal images, through a non-destructive evaluation [1]. PTM allows the acquisition of thermal and optical images of samples with inhomogeneous structures such as agricultural seeds. PTM techniques include, among others, photoacoustic microscopy (PAM) and photopyroelectric microscopy (PPEM).

PAM has been successfully used for surface and sub-surface analysis of solid materials. The photoacoustic (PA) effect is the physical basis for PAM imaging, i.e., it refers to the generation of acoustic waves by the absorption of electromagnetic energy, such as optical or radio frequency. Alexander Graham Bell first reported the observation of sound generated by light in 1880 [2]. In a PAM experiment the modulated light beam, absorbed by the sample, is converted into acoustic or elastic energy via the conversion of light into thermal energy [3]. The technique involves a modulated laser beam which is focused, to scan the sample of interest. The thermal waves generated in the sample, by the periodic heating, scatter defects below the surface of the sample, and this causes a change in the PA signal. As the laser beam is scanned across the sample, any change in the PA signal indicates the presence of defects or thermal property variations at that location [4].

Imaging by PAM has been successfully performed for several materials and structures; however, this technique has rarely been applied to biological samples [3], such as agricultural seeds.

Seed quality is an important criterion of purchase, and it is a specific requirement because it affects crop establishment, growth, and yield; therefore, it has an important influence on agricultural production [5]. In many Latin–American countries, maize (*Zea mays* L.) and wheat (*Triticum aestivum* L.) are the basic crops, where physiological quality plays a fundamental role in the achievement of adequate seedling emergence and crop establishment [6]. In order to obtain higher crop potential in yield and/or quality, seeds of high quality to produce rapid and uniform seedling emergence are required [7]. Seed vigor is the physiological quality component that is closely related with rapid, uniform emergence, and development of normal seedlings (under a wide range of soil conditions) [8]; also, factors such as ageing contribute to seed quality. By considering that a real problem in the seed industry is the evaluation of seed quality [9], the development of non-destructive techniques to evaluate seeds through PA images would be very useful. In this sense the PAM technique, which is a

non-destructive method, could be used in the study of seeds. Therefore, the main objective of the present study was to obtain PA images of wheat and maize seeds, from different productive cycles (i.e., from different annual cycles). The analysis has shown that PAM is an alternative technique to evaluate seed deterioration. Also, optical absorption spectra of these seeds were obtained by photoacoustic spectroscopy (PAS).

2 Materials and Methods

2.1 Biological Material

Maize seeds from cross CL-12 \times CL-11 (Genotypes: produced in 2005 and 2007) and wheat (Genotypes: produced in 2005 and 2007) have been provided by the Mexican Genetic Quality Control Assurance Institute (IREGEP) for the present research project.

2.2 Experimental Setup

2.2.1 PAM Technique

A schematic diagram of the experimental setup used for PAM technique is shown in Fig. 1. In this mounting the PA cell and its sensor, an electret microphone, are mounted on an $x-y$ motorized stage, with spatial resolution of $50\ \mu\text{m}$. The excitation source is a fiber coupled laser diode, at 650 nm wavelength, modulated in intensity at 1 Hz frequency, by the reference oscillator of the lock-in amplifier. By using a microscope objective, the laser beam was focused on the seed surface. The PA signal was pre-amplified and sent to the lock-in amplifier. A personal computer was used to control

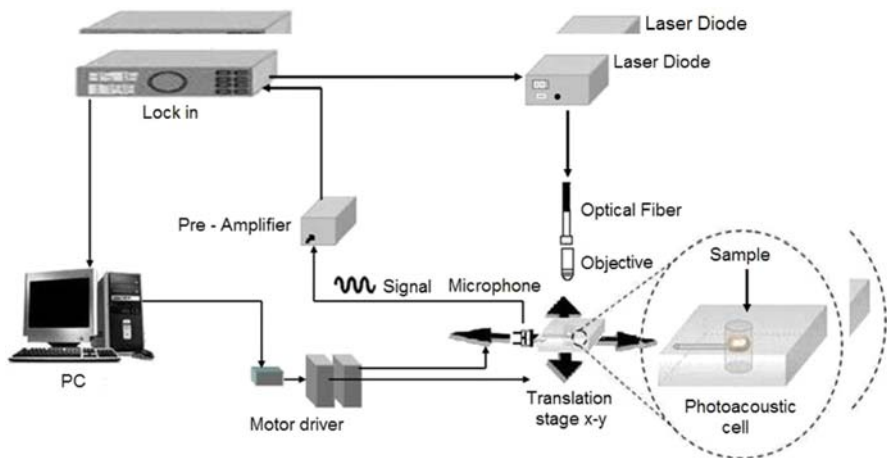


Fig. 1 Schematic diagram of the experimental setup used for PAM technique

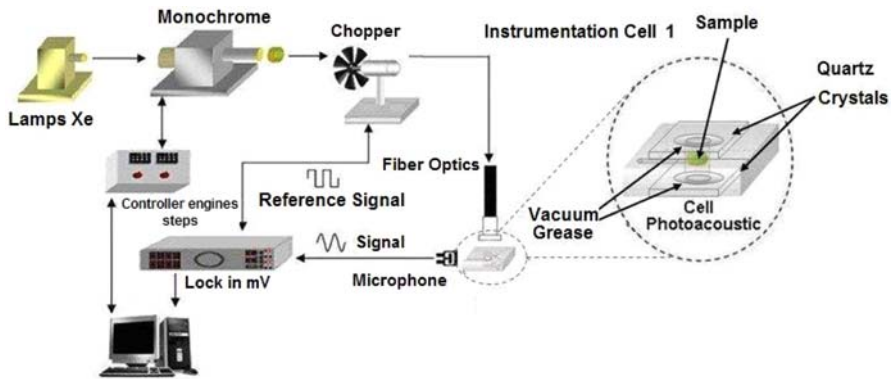


Fig. 2 Experimental setup of the photoacoustic spectrometer used to obtain the optical absorption spectrum of wheat and maize seeds

the scanning of the $x-y$ stage and also to record, from the lock-in amplifier, the experimental PA signal amplitude and phase from each point of the scanned sample. Wheat and maize seeds were placed inside of the PA cell, and their PA signal was recorded as a function of its position in order to obtain a PA image of each seed.

2.2.2 PAS Experimental Setup to Obtain the Optical Absorption Spectra of Seeds

In order to see also spectroscopic differences between the same groups of seeds, used in the PAM technique, their optical absorption spectra, ranging from 350 nm to 800 nm, were obtained by using PAS. The PA experimental setup consists of a 1 kW Xe lamp (Oriel), a mechanical chopper operating at 17 Hz, a monochromator (Oriel), and a homemade brass PA cell provided with an electret microphone. The PA signal from the microphone was fed into the input channel of a SR-850 (Stanford Research) lock-in amplifier interfaced to a personal computer that displays simultaneously both the amplitude and phase of the wavelength-dependent signal (see Fig. 2).

3 Results and Discussion

Figure 3 shows the photothermal images of wheat and maize seeds, from different productive cycles. The measured areas were 4 mm \times 6 mm and 6 mm \times 6 mm for wheat and maize, respectively.

Figure 3 shows the results of the thermal imaging of wheat and maize seeds. In this figure it is possible to see three columns; i.e., the first refers to the optical image; the second shows the PA image; and the third shows the PA contour image. The PA images shown in the third column have a color scale which corresponds to differences in the PA signal due to different components in both wheat and maize seeds, and also for younger and older seeds. The PA contour image (see third column) has advantages when compared with the optical images (see first column). That is, the PA contour image provides a clear difference in optical and thermal properties in different regions of the samples when comparing younger and older seeds.

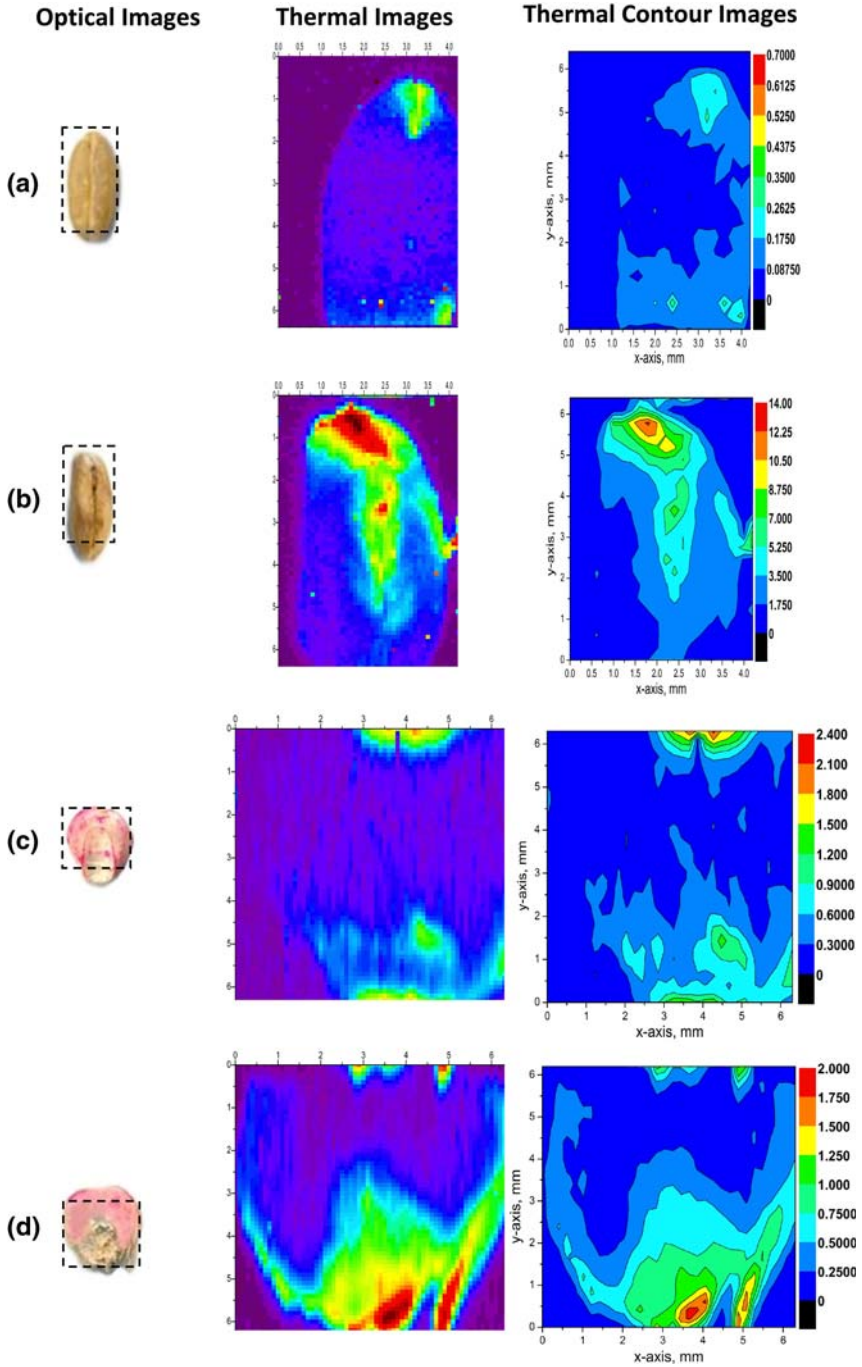


Fig. 3 Photothermal images: (a) wheat seed produced in 2007, (b) wheat seed produced in 2005, (c) maize seed produced in 2007, and (d) maize seed produced in 2005

The seeds are composed of a variety of materials with different thermal and optical properties. These properties change over time and cause a degradation leading to seed ageing. In the present study these changes have been sensed by the obtained PAM images where the PA signal was generated from each point of the sample in the scan process.

Figure 3a shows the optical and PAM images obtained from wheat seeds produced in 2007. It can be seen in PAM images the uniformity of the image, which is due to homogeneous light absorption resulting from the uniform color in the seed and also the homogeneity in the seed structure and thermal properties.

Moreover, the PAM image of an older wheat seed (produced in 2005) shows marked inhomogeneous regions (see Fig. 3b) caused by the differences in coloration and structure due to deterioration of this seed in comparison with the younger wheat seed. It is shown in the image a strong difference in brightness intensities with respect to the younger seed due to the deterioration which could be due to the storage time and also by mycoflora associated to the seed. The importance of the PAM image is mainly due to the fact that the deterioration for some type of seed disease could be observed to evaluate the health of the seed.

The optical and PAM images of maize seeds produced in 2007 and 2005 are showed in Fig. 3c and d, respectively. Optical images show some differences between the different cycles; however, the PAM images showed more differences in brightness when comparing the older seed with the younger one. Also, in the PAM contour image, it is possible to quantify the brightness differences due to seed deterioration.

The present study on the assessment of damage of wheat and maize seeds, by using the PAM technique, is a direct and non-destructive evaluation; although the technique takes some time, this method shows that it is possible to incorporate new techniques in agricultural projects for evaluation of seeds.

Also we observed for both seeds that the PA signal is higher in the seed with more deterioration; this could be due to the porosity of the material and the different color of the seed, since the PA signal is dependent on the thermal and optical properties of the sample. The images show more deterioration in embryo for both seeds, it is possible that the deterioration began in this part of the seed for both samples.

This result suggests that changes in the intensity of the image, when the seed has some deterioration, will allow determination of whether the seed is alive or dead, in short time, depending on the imaging resolution. Abnormal seeds gradually lose vigor, before they lose the capacity to germinate and finally die. Seed infection or age deterioration may lead to low germination, reduced field establishment, severe yield loss, or a total crop failure [10].

On the other hand, optical absorption spectra of these seeds were obtained by using PAS. Figure 4a shows optical absorption spectra of wheat seeds, where it is possible to see lower optical absorption in the young seed when compared with the older one. This could be due to deterioration in the older seed because of the presence of fungi or bacteria during storage, and this fact produces dark regions and, as a consequence, a higher optical absorption. Figure 4b shows PA optical absorption spectra of maize seeds of different production cycles. Both seeds were in storage conditions, where a fungicide is applied, and therefore, these seeds show coloration due to this fungicide. The younger seed, without deterioration, shows an absorption band, in the

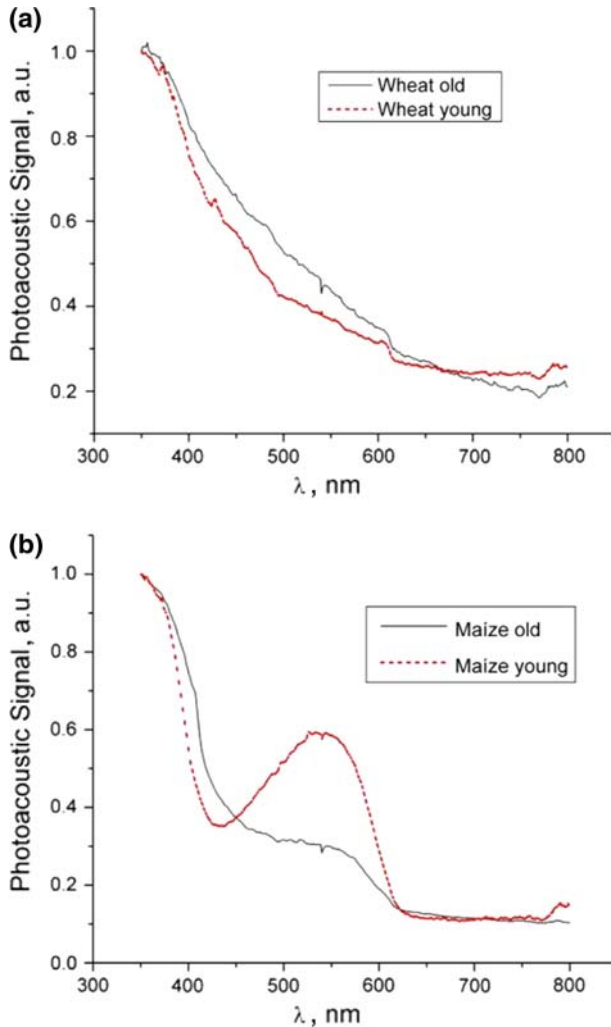


Fig. 4 PA signal amplitude: (a) wheat seeds old and young and (b) maize seeds old and young

445 nm–610 nm range, due to the coloring of the fungicide in the pericarp, which is less deteriorated when compared with the older seed. The spectrum of the older seed shows less absorption in this band because the dye in the seed also diminished with time.

The differences in the optical and thermal properties in young and old seeds can be observed in the optical absorption spectra, obtained by PAS and PAM images. In the case of the younger seed samples, a more homogeneous image is obtained when compared with the PAM image of the older seeds for which the image shows a less homogeneous distribution due to seed deterioration. The PAM technique allows us to observe differences in biological materials such as seeds of maize and wheat in different periods of storage.

The results show that the PT techniques, in this case the PA technique, makes it possible to evaluate the surface quality of wheat and maize seeds. Seed quality depends on genetic components (defined by the variety), physical purity, viability, and seed health (disease). Seed tests provide information to determine the quality of seeds [10]. This information is needed to reduce the risks resulting from planting poor quality seeds. Seed tests are reported in several publications of the Association of Official Seed Analysts [11] and the International Seeds Test Association [12], but for several of these tests, it is not possible to use them due to the fact that the analysis uses chemical substances and the process itself could damage the seeds which disables their use.

4 Conclusions

Photoacoustic microscopy techniques provide possibilities to evaluate seed quality due to: (a) no special sample preparation is required to obtain PA images; (b) PT techniques are non-destructive methods, and it is possible to use them to determine seed quality for sowing; and (c) PAM techniques can be used to observe differences in biological materials such as seeds of maize and wheat in different periods of storage.

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