

Depth Profiles in Maize (*Zea mays* L.) Seeds Studied by Photoacoustic Spectroscopy

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Abstract Photoacoustic spectroscopy (PAS) has been used to analyze agricultural seeds and can be applied to the study of seed depth profiles of these complex samples composed of different structures. The sample depth profile can be obtained through the photoacoustic (PA) signal, amplitude, and phase at different light modulation frequencies. The PA signal phase is more sensitive to changes of thermal properties in layered samples than the PA signal amplitude. Hence, the PA signal phase can also be used to characterize layers at different depths. Thus, the objective of the present study was to obtain the optical absorption spectra of maize seeds (*Zea mays* L.) by means of PAS at different light modulation frequencies (17 Hz, 30 Hz, and 50 Hz) and comparing these spectra with the ones obtained from the phase-resolved method in order to separate the optical absorption spectra of seed pericarp and endosperm. The results suggest the possibility of using the phase-resolved method to obtain optical absorption spectra of different seed structures, at different depths, without damaging the seed. Thus, PAS could be a nondestructive method for characterization of agricultural seeds and thus improve quality control in the food industry.

Keywords Depth profile · Modulation frequency · Phase-resolved method · Photoacoustic spectroscopy

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1 Introduction

In the food and seed industries it is important to evaluate the quality of the seed and, often for its evaluation, destructive methods are used. In this sense a nondestructive method, photoacoustic spectroscopy (PAS) combined with the Rosencwaig and Gersho model has been successfully used to obtain the optical absorption coefficient of agricultural seeds and food [1–4]. Other studies with the PAS technique have investigated healthy and infected (with *Colletotrichum gloeosporioides*, fungus) acai seeds (*Euterpe oleracea*) [5]. Photoacoustic spectroscopy has also been shown to be a sensitive technique to characterize inhomogeneous materials. For example, studies of optical characterization by PAS in maize grains of different varieties used in tortilla and dough industries have been validated by comparing the PAS results with those obtained from a UV–Vis spectrophotometer with an integrating sphere [6]. Also, by using PAS, it was possible to obtain differences due to the color of maize seeds by obtaining the optical absorption coefficient of these seeds with different pigmentations. Added to these potential benefits of PAS, it has also the ability to obtain information about the depth profile of the sample [7].

An agricultural seed is the part of the fruit phanerogams containing the embryo of a future plant, protected by a “testa.” There are different varieties of seeds for agricultural production, which can be incorporated into plant breeding over time (different production cycles). The seeds are composed of different layers depending on the variety or genotype: the surface layer is the pericarp, followed by the aleuronal layer (where in some cases the pigment is found). These two layers cover different types of endosperm (floury and crystalline) constituting the interior of the seed. Furthermore, a fifth structural component, which is the germ, can be observed, with these layers being the most representative in the seeds.

The structure of the seed can be modified according to its quality, and the seed quality depends on genetic components, its physical purity, its viability, and its seed health [8]. Seed tests provide information to determine the quality of seeds, and 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 [9] and the International Seeds Test Association (ISTA) [10], but for several of these tests, it is not possible to use the seeds once again due to the fact that the sample seeds are damaged by some chemical processes. Other authors have reported methodologies to evaluate agricultural seeds by means of their images. Among these methods, radiographic analysis by X-rays (ionizing radiation) is included, which is one of the methods approved by the ISTA (1996) [11], to evaluate seed quality. However, the use of X-rays to identify if the seeds are full, empty, or have changes in their morphology to evaluate the physical and physiological seed quality through feasibility tests, can generate internal damage [12, 13]. PAS can be used to perform characterization of the optical absorption, from which an indirect measure of color, could be used to identify the differences between the absorption bands due to different pigments contained in diverse varieties or genotypes of seeds. Then, the objective of this research was to obtain optical absorption spectra of maize seeds (*Zea mays* L.) by means of PAS, at different light modulation frequencies (17 Hz, 30 Hz, and 50 Hz) and compare these spectra with those obtained from the phase-resolved method in order to obtain the

depth profiles of the optical absorption spectra of maize seeds. Thus, PAS could be a nondestructive method for characterization of agricultural seeds and thus improve quality control in the seed industry.

2 Materials and Methods

2.1 Biological Material

Maize seeds from HA-13E, produced at the INIFAP, Montecillo, Mexico, during the spring–summer period of 2012, were used. The seed genotypes had bluish pigmentation; the physical dimensions and nutrient content of maize seeds used in this investigation are shown in Table 1.

2.2 PAS Experimental Setup

Figure 1 shows the PAS experimental setup which consists of a xenon lamp as the light excitation source, a monochromator to obtain a monochromatic light beam, and a mechanical chopper to modulate the light beam at a fixed modulation frequency $f = 17$ Hz. The modulated beam was focused onto an optical fiber in order to guide this beam to the photoacoustic (PA) cell. The acoustic signal generated in the PA cell was detected by a microphone through a fine channel (diameter, 1 mm) between the PA cell and the microphone inlet. Then the PA signal was amplified using a lock-in amplifier (EG&G, 5210) and interfaced with a personal computer. The PA signal was recorded as a function of wavelength of the modulated light beam.

In order to obtain the optical absorption spectra of the entire maize seed samples, a new PA cell was made. The new PA cylindrical cell had a diameter of 16 mm and was 9 mm high. The obtained photoacoustic signal was pre-amplified before being sent to the lock-in amplifier.

The entire maize seed is placed in the PA cell as shown in Fig. 2, and the optical absorption spectra, in a wavelength range from 330 nm to 800 nm, were obtained by PAS at three light modulation frequencies: 17 Hz, 30 Hz, and 50 Hz.

3 Results and Discussion

For this study, as shown in Fig. 1b, the modulated beam was focused on the dorsal side of the maize seed (with the germ in the opposite side). The seed was placed in the photoacoustic cell between two quartz windows, sealed with vacuum grease, at the bottom and top of the opening. Keeping in mind the irregular geometry of the seed, we selected seeds with the most regular shapes (flat ones) so that the light beam impinges on the surface of the sample.

By varying the modulation frequency of the light beam, the photoacoustic spectra shown in Fig. 2 were obtained. In this figure, it is possible to observe the amplitude and phase spectra obtained by using different modulation frequencies. As was expected, the intensity of the PA signal decreases as the modulation frequency increases.

Table 1 Physical dimensions and nutrient content of maize seeds

Variety	Physical dimensions				Nutritional content						
	Productive cycle	Thickness (mm)	Length (mm)	Width (mm)	Moisture (%)	Humid base			Dry base		
						Ashes (%)	Crude fiber (%)	Protein (%)	Ashes (%)	Crude fiber (%)	Protein (%)
1	2012	5	15	9	8.8	1.3	2.0	7.5	1.4	2.1	7.6

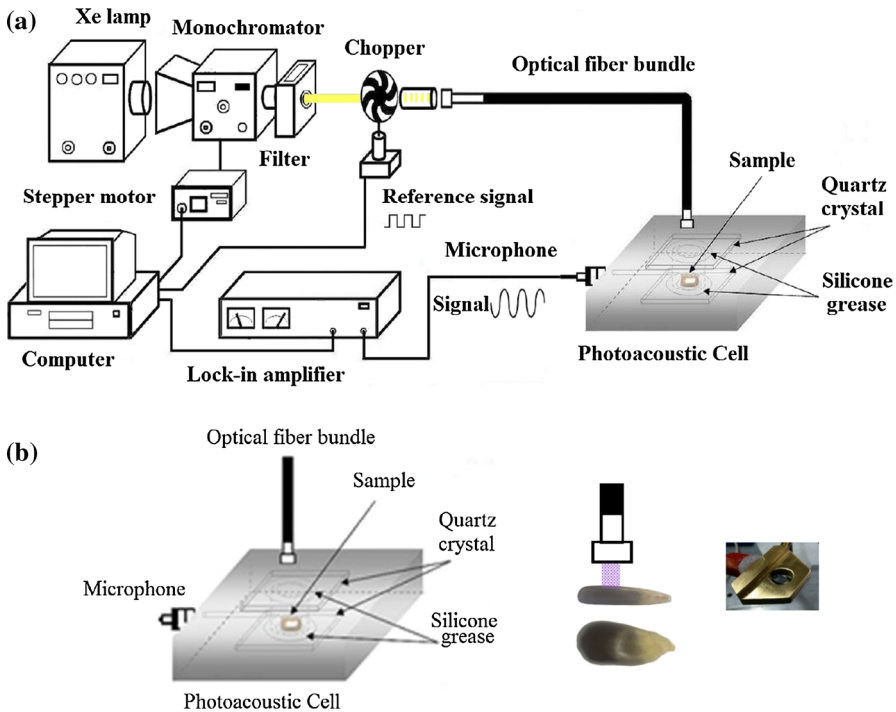


Fig. 1 (a) Experimental setup for PAS measurements (1—xenon lamp, 2—monochromator, 3—chopper, 4—photoacoustic cell, 5—lock-in amplifier, and 6—PC) and (b) photoacoustic cell (volume of 1.8 mm^3)

The analyzed maize seed had blue pigment in the aleurone layer and, consequently, a high optical absorption was obtained from this sample. On the other hand, it is possible to perform the analysis of different depth profiles, which are related to the different layers where several structural components of the sample are present. An analysis of different depth profiles by the PA signal is possible using the phase-resolved method (PRM) for several layers. It is also possible to see (Fig. 2b) that the phase of the photoacoustic signal presents several absorption bands, which can be attributed mainly to the thermal properties of the seed components.

Around 450 nm, the optical absorption spectra have an absorption band which is better observed at low frequency (17 Hz), and this band tends to disappear when the light modulation frequency is increased (at 30 Hz and 50 Hz). The spectrum at 17 Hz (lower frequency) would be related to greater depth penetration, and this is the response of endosperm tissue. In Fig. 2a, a wide absorption band ranging from 550 nm to 750 nm can be attributed to the anthocyanins in the aleurone layer. However, when the light modulation frequency is increased, this absorption band diminishes and only the optical absorption spectra of pericarp are observed. The spectrum obtained at 30 Hz is closer to the sample surfaces, where the absorption band between 530 nm and 700 nm showed an increase, due to pigment content in the aleuronal layer: the anthocyanins for the blue maize with $(35 \pm 10) \mu\text{m}$ thickness. Concerning the spectra obtained at the frequency of 50 Hz, it could be related to the surface layer of the sample: the pericarp, with a thickness around $(65 \pm 5) \mu\text{m}$.

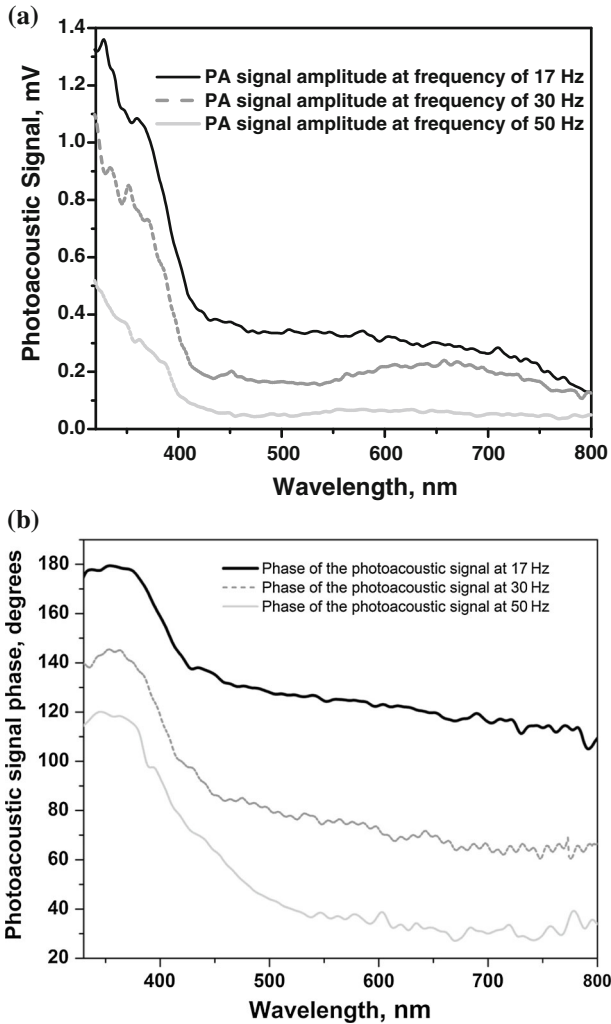


Fig. 2 Experimental photoacoustic signal of maize seed at different modulation frequencies 17 Hz, 30 Hz, and 50 Hz (a) amplitude and (b) phase

On the other hand, it is possible to observe PA signal decomposition due to the different components in the maize seed, in this case the pericarp and aleurone layer, which are located at different depths, with the pericarp as the outer layer. Considering this sample as a two-layer system (pericarp as layer A and aleuronal as layer B), the composed PA signal S_{PA} is, therefore, obtained as a sum of the corresponding vectors to each layer (S_A and S_B). Both vectors are out of phase by $\Phi = \phi_A - \phi_B$. The phase-resolved method uses the photoacoustic spectra and provides a composition for several phase angles according to Quiroz et al. [14]:

$$S(\lambda) = |S_\lambda| \cos(\theta - \phi_\lambda). \quad (1)$$

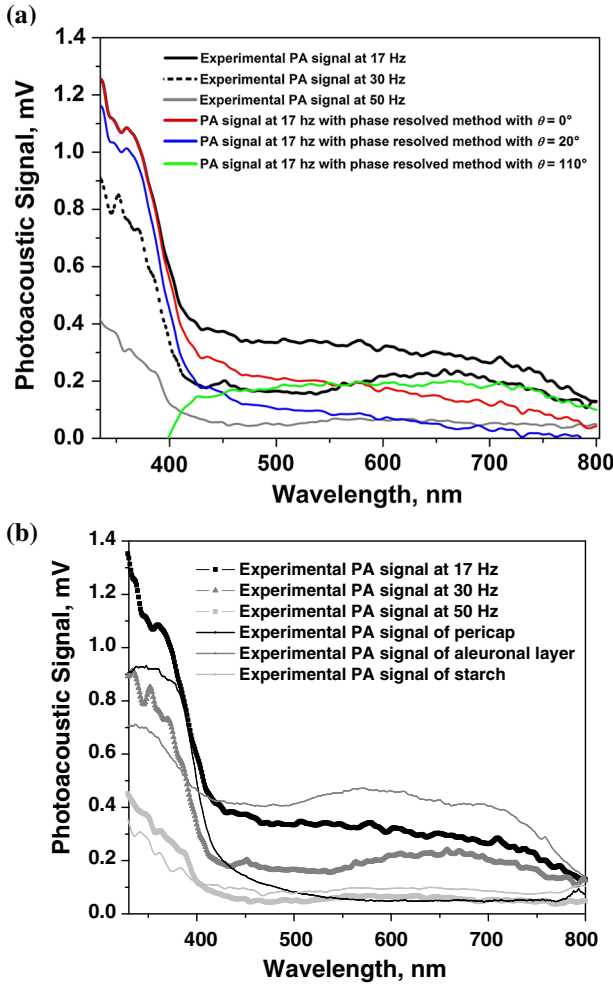


Fig. 3 (a) Optical absorption spectra obtained by PAS at different light modulation frequencies and phase-resolved method applied to the PAS optical absorption spectra and (b) optical absorption spectra obtained by PAS at different light modulation frequencies and PAS absorption spectra of separate seed structural components

In this case, with a single experiment of PAS at one frequency (17Hz), it is possible to obtain the separated spectra of each layer. With a PAS experiment at one frequency (17Hz), the separated spectra of each layer can be obtained. Applying the theoretical model, by using Eq. 1 to the experimental data, it can be seen in Fig. 3a, that at $\theta = 0^\circ$ in Eq. 1, the obtained spectrum is similar to those obtained at 30Hz. At $\theta = 20^\circ$ in Eq. 1, the calculated spectrum is similar to the experimental spectrum obtained at 50Hz, which corresponds to the most superficial layer (pericarp of the maize seed) according to Muñoz et al. [15].

Finally, when $\theta = -110^\circ$, the obtained spectrum is similar to the wide absorption band ranging from 550 nm to 750 nm due to the anthocyanins in the aleurone layer.

Photoacoustic spectra, of the separate structural components of the seed, were obtained (Fig. 3b). As shown in this figure, photoacoustic spectra of the structural components of the seed (pericarp, aleuronal layer, and endosperm), presented the same behavior of the spectra obtained from the whole seed by varying the modulation frequency. Thus, the PAS technique allows depth profile analysis on complex specimens with different structural components. On the other hand, using PAS, it is possible to study the absorption centers of the pigment associated with each structural component.

In the present research, we have performed a nondestructive study, using PAS, varying the modulation frequency, as well as using the phase-resolved method to characterize the optical absorption pigments of maize seeds with natural blue pigments. These results support the capability of the PAS technique to perform a study of the internal components by changing the modulation frequency or also by the phase-resolved method without destroying the seed. For a real-world application samples of (200 or 400) seeds of a lot of these are taken to determine their physiological quality, according to the rules of ISTA or AOSA. Future studies could lead to the construction of a photoacoustic cell where several seeds can be placed and be useful for the seed industry or seed testing laboratories.

Ideally for a farmer, if 100 seeds are sown, the 100 seeds can be set and bear fruit. Thus, adequate physiological seed quality is necessary to optimize the resources invested by a farmer. Seed quality can be subdivided into four basic qualities: genetics, health, physical, and physiological. The presence of the four essential qualities at the maximum levels allowed corresponds to having seeds of the highest integral quality. Each of them contributes its part to generate productive plants. Weakness in any of them introduces a limiting factor and, as a consequence, generates plants with low productivity. In order to achieve an increase in productivity, it is necessary to have good physiological quality seeds [16], having the ability to germinate, emerge, and lead to uniform and vigorous plants.

This study adds to other studies that show that the PAS technique has the sensitivity to distinguish differences between spectra obtained from different seed varieties. Also, in this study we demonstrated that it is possible to observe through their optical absorption spectra, structural layers within the seed at different depths. Future research will be directed to explore the possibility, in a wide variety of seeds, to identify and obtain their optical absorption spectra at different depths.

4 Conclusions

This research demonstrates that by using PAS it is possible to identify different components within the seed, through their optical absorption spectra, obtained at different modulation frequencies and also by the phase-resolved method. Finally, these obtained spectra were compared with the spectrum of each seed component separately.

It was shown that the optical absorption spectra in the region of 330 nm to 800 nm was constituted by the superposition of three absorber layers, one of them corresponding to the pericarp layer and the others corresponding to the aleurone layer and the endosperm.

The obtained optical absorption spectra, at a 17 Hz modulation frequency, has two main absorption bands, at 450 nm and another in the range between 550 nm and 750 nm, corresponding to the natural blue pigments in the aleurone layer.

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