Characterization of seeds with different moisture content by photoacoustic microscopy

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Abstract. Photoacoustic (PA) technique has important applications for material characterization and nondestructive evaluation of opaque solid materials. PA microscopy allows the acquisition of information of samples with inhomogeneous structures as agricultural seeds. A determining factor for seed safe storage is their moisture content. Seeds stored at high moisture content exhibit increased respiration, heating, and fungal invasion resulting in poor seed vigor and viability. Low moisture content, in the seed to be stored, is the best prevention for these problems. In this study, Photoacoustic Microscopy (PAM) was used to characterize seeds with different moisture content. In the PAM experimental setup the photoacoustic cell and its sensor, an electret microphone, are mounted on an x-y stage of mobile axes, with spatial resolution of 70 μm. The excitation light source is a fiber coupled laser diode, at 650 nm wavelength, modulated in intensity at 1 Hz of frequency, by the reference oscillator of a lock-in amplifier. By using a microscope objective the laser beam was focused on the seed surface. The resolution was enough to obtain differences in the obtained images, which are dependent on the moisture content. This method, to study differences in the seed moisture content, is nondestructive and could be useful for a sustainable Agriculture.

Keywords: Photothermal microscopy (PTM), Photoacoustic microscopy (PAM), seed moisture content.

1. Introduction.
Photoacoustic microscopy (PAM) has important applications in material characterization and nondestructive evaluation of opaque solid materials (1). PAM technique allows the acquisition of information of samples with inhomogeneous structures as agricultural seeds (2). The principle of PAM technique is based on the generation of thermal waves, as a result of the absorption of modulated light on the sample. When light penetrates through biological samples, some portion of the light is absorbed and transformed into heat. The rapid heat transform produces a temperature variation in the corresponding period, the generated thermal wave induce a periodical heating in the sample and as a consequence a pressure variation in the gas within the closed photoacoustic (PA) cell. These pressure waves can be measured in each point of the sample by scanning the focused light beam on the sample surface and reconstructed to form a PA imaging that represents the local sources and distributions of
the optical absorbers in the sample. A sensitive microphone is used to detect the pressure variations in the PA cell.

The PAM technique allows obtaining images which are dependent of the optical and thermal properties of the sample. On the other hand the thermal properties of seeds are essential in their storage. An important factor for seed safe storage is the seed moisture content.

Seeds stored at high moisture content exhibit increased respiration, heating, and fungal invasion resulting in poor seed vigor and viability; low moisture content in the seed, to be stored, is the best prevention of these problems (3). In the case of agricultural seeds the increase of moisture content is an important factor in seed viability preservation due to the role of water in the activity of the metabolic processes which determine its vigor and longevity, and also allow the development of insects and storage fungi (4, 5).

Changes in the seed moisture content induce variations in its thermal properties. Aviara and Haque (2001) and Yang et al. (2002) evaluated the thermal diffusivity of pigeon pea, gram, soybean, sheanut kernel and borage seeds, and they observed that thermal diffusivity of these products increased with the increase of moisture content. Aviara et al (2008) reported that thermal diffusivity of ground seed and kernel decreased with the moisture content.

The radish and tomatoes are excellent sources of vitamins and minerals in the diet of Latin-American persons. So, the seeds should be stored properly and then it is important to know their thermal properties. By making a review of the literature we do not found any correlation between the thermal properties of radish (Raphanussativus) and tomato (Lycopersicon esculentum) seeds with their moisture content. The aim of this study was employed PAM technique to characterize radish and tomato seeds with different moisture content.

2. Materials and methods

2.1 Biological Material. Radish and tomato seeds, for the present research project, have been provided by the Protected Horticulture Area of the Autonomous University of Chapingo, Mexico. The seeds were classified according to their size, shape and color. The average weight of twenty radish and tomato seeds were 0.1767 g and 0.0229 g respectively.

The seed weights were obtained by using an analytical balance (Adventurer-Ohaus). The initial moisture contents were 2.52% and 3.76% for radish and tomato respectively. Then the radish and tomato seeds were soaked during 30 and 5 seconds respectively and their moisture content were 11.94% and 18.54%. The gravimetric method was used to obtain the moisture content. The measurements are performed for duplicate, to obtain the average values of the moisture content for each sample seed (radish and tomato).
2.2 PAM Experimental Set-up.
The PAM experimental set-up is shown in Figure 1. In this set-up the photoacoustic cell and its sensor, an electret microphone, are mounted on an x-y motorized stage, with spatial resolution of 70 μm. 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 a lock-in amplifier. By using an objective of microscope, 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 the x-y stage, in order to scan the focused laser beam on the sample surface, and also to record, from the lock-in amplifier, the experimental PA signal amplitude and phase. Radish seed (dry and after soaked) was 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. This procedure was repeated for tomato seeds.

![Figure 1 PAM experimental set up](image)

The PAM images of radish and tomato seeds at room humidity conditions and increased humidity were obtained.

3. Results and discussion
Figure 2 shows the photoacoustic images of radish and tomato seeds, from different moisture content. The measured areas were 3.5 x 3.5 mm and 4 x 5 mm for radish and tomato seeds respectively.
Figure 2. PA images a) dry radish seed, b) soaked radish seed, c) dry tomato seed and d) soaked tomato seed

Figure 2 shows the PA imaging for the case of radish and tomato seeds. It can be seen three columns, the first one refers to the optical image; the second one shows the PA image; and the third one shows the PA’s contour image. The PA images, in the third column, show in a scale of colors the differences in the PA signal due to different components in both radish and tomato seeds, and also for seeds of different moisture content. 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 both radish and tomato seeds, and for dry and soaked seeds.

A homogeneous PAM images for dry radish and tomato seeds were obtained but in the case of these seeds, with increased moisture, the PAM images show different regions due to the water absorption in the seeds which could cause local differences in moisture content which also change their local thermal properties.
Others authors found variation in thermal properties in other seeds as a function of their moisture content (6-9). In the case thermal diffusivity, this property increases or decreases, depending of the variety of seed, with the increase of moisture percentage.

An increase in moisture content promotes the enzymatic activity which is important when the seeds are stored. Less moisture content in storage ensures a higher pathological and physiological seed quality.

4. Conclusions
PAM is a sensitive technique to observe changes in the thermal properties in radish and tomato seeds due to differences in moisture content, this is an important factor for seed storage. In the present research differences in the PA images were found in radish and tomato seeds at different moisture content (2.52% and 3.76% al 11.94% and 18.54% for radish and tomato respectively). Changes in the moisture content of seed, leads to changes in PA images.

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