Carotenoids and Flavonoids identification in Brazilian Tropical Fruits and Vegetables using Photoacoustic Technique

Rodrigo José da Silva Lima, José Suassuna Filho

Av. Aprígio Veloso, 882 – Campina Grande. Universidade Federal de Campina Grande, Departamento de Física – Centro de Ciências e Tecnologia, Laboratório de Espectroscopia Fotoacústica

Rodrigo@df.ufpb.br

Abstract

There has been increasing progress in the Photoacoustic Spectroscopy (PAS) as a powerful analytical technique in material science. The PAS measures a sample absorbance spectrum directly with a controllable sample depth and with little or no sample preparation. The PAS analysis is applicable to nearly all kind of sample in a non-contact and non-destructive way in spectral range from UV to far infrared. In this work we present results of the application of PAS technique to the analysis and identification of biomolecules in a variety of biomaterials such as plant leaves, fruits and vegetables.

Photoacoustic spectra were detected in a wide variety of samples and were associated **carotenoids** and **flavonoids** molecules in leaves, fruits and vegetables. The results suggest that PAS can become a rapid direct and efficient analytical method in material science, particularly in the very promising field of photochemistry and photobiology.

Introduction

The β-Carotene and Lycopen are the main representative biomolecules of the carotenoid configurations photosynthetic complex well known as protector against photo induced damage. They are naturally occurring compounds in plants, fruits and vegetables.

They have several functions in humans, including protecting against the action of free radicals. In addition, a low level of β-Carotene is associated with a poor cognitive performance, and higher β-carotene levels are associated with better memory performance in elderly people. Carotenoids have been postulated also to play a protective role against cardiovascular disease and cancer. All the positive effects on health postulated for the carotenoids have been attributed largely to their antioxidant actions.

The flavonoids (or bioflavonoids) represent the major group of non-nutrients that accumulate in epidermic cells of plants and provide a barrier against UV radiation damage. It has been postulated that the quercetin (a flavonoid-containting compound) inhibits the proliferation of human carcinogenesis cells and reduces the development of UV-induced tumors. Therefore, fruits and vegetables riches in bioflavonoids are anticarcionogenic as well as anti-inflammatory compounds. A number of studies has consistently shown that a high consumption of fruits and vegetables reduce the risk of cancer at most sites other then prostate. It was proposed that the delay in the onset of tumor growth and even the suppression of cancer observed in experimental groups of individuals, may be due to leak of nutrients like protein and fat in bioflavonoid compounds.

Certain flavonoid inhibit the adhesion of fat platelet on the arterial walls and seems to be a potent active inhibitor of the arteriosclerosis as determined by a large variety of in vitro and in vivo observations.

We have conducted investigation to detect the presence of carotenoids and bioflavonoids in fruits and vegetables using Photoacoustic Spectroscopy (PAS). The primary aim of our study is to introduce a fast, sensitive and non-destructive physical technique to select food products riches in biomolecules that play important role in improvising health quality, as the β-carotene, lycopene and bioflavonoids.

The main goal of our study was identify the flavonoid biomolecule (quercetin) in several kinds of fruits, leaves and vegetables from PAS spectra as seen bellow. This could lead to a method of selection and manipulation of vegetables and fruits of strategic nutritive value by PAS spectral analysis.

Experimental Setup

The potential of photoacoustic spectroscopy (PAS) as a powerful method to investigate any material phase (gas, liquid, semisolid and solid) has already been demonstrated[(1),(2),(3)]. In particular, PAS measurements of solid samples has proved to be useful for determination of some of their optical our thermal properties such as the optical absorption coefficient β, the thermal diffusion length and thermal difusibility. In our effort to investigate a wide variety of materials like polymers, composites, biological and minerals, we have mounted a photoacoustic spectroscopy laboratory, briefly described below. Fig.1 shows the block diagram of our photoacoustic spectrometer.

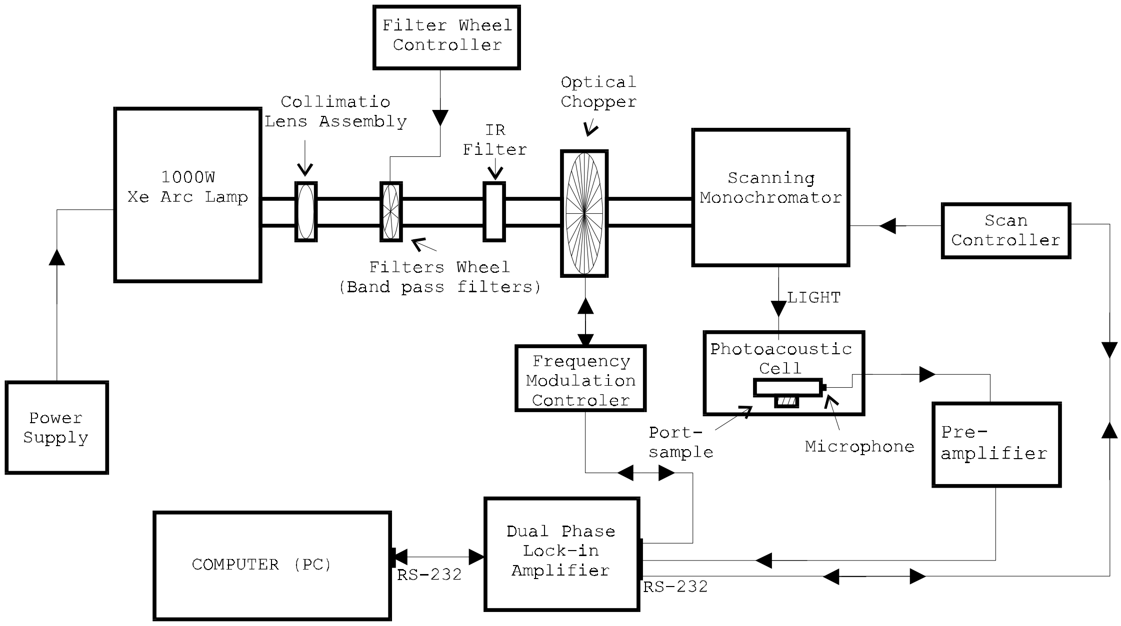

Fig.1 - Block Diagram of the Photoacoustic Spectrometer, Department of Physics, CCT, Federal University of Campina Grande, Pb (Brazil).

Our PAS system is a high-energy spectrometer. The light source is a 1000W-ozone free xenon arc lamp with a wide spectral range, IR-VIS-Ultraviolet. The radiation, after having passed a water filter to remove excess of heat (IR radiation) and by a five position automatically controlled filter wheel to eliminate the radiation multiple order effects, enter to a chopper modulation system that provide the modulation of the light beam. This chopper is computer controlled and allows continuous frequency scanning from 0 to 2KHz, or discrete frequencies values, allowing the detection of PAS spectra as a function of the wavelength. The modulated beam is then expanded to the entrance slip of the monochromator. A 1710 G/mm holographic diffraction grating (mod. 1710 from the McPherson) provides a high efficient monochromatic light within the Visible-UV region with a high accuracy of ± 0.7nm and resolution of ≈1.0nm. A computer controlled high-resolution (25000 steps/s) scan controller unit, using a standard RS-232 operates the spectrometer. A focusing exit lens assembly directs the beam 90º downward to hit the sample inside the photoacoustic detector. This system incorporates a two-phase sensitive detector (PSD) lock-in amplifier (mod. SR750 from the Stanford Research), phase electronics and a manipulative software (grams/386). The spectrometer control, acquisition and manipulation of the data are performed by a Macintosh and Windows compatible platform.

Results and Discussions

Photoacoustic spectroscopy experiments were carried out on several plant leaves, fruits and vegetables in order to try identify the carotenoids and bioflavonoids biomolecules in those species. Fig.1 shows a representative PAS spectrum of a commercial concentrate of carotenoids where appear several peaks identified as being of the β-carotene in two configurations (425; 451; 478)nm at high concentration and (427; 455; 482)nm at very low concentration, neoxathin (415; 437; 466)nm , rubixanthin (432; 462; 494)nm and lycopene (445; 472; 502)nm. Identification was possible by comparing the observed PAS spectra with the conventional optical absorption spectra of the several investigated biomolecules found in the literature. Fig. 2 shows the PAS spectra obtained for the carrot where the β–carotene peaks were promptly observed. In addition a set of peaks was detected and associated with others carotenoid configurations as follow: lycopene (445; 472; 505)nm and (456; 485; 520)nm ; lutein (421; 445; 475)nm and finally a set of peaks (417; 440; 469)nm that coincide with the optical absorption peaks present in the lutein 5,6 epoxide. The lutein configurations belong to the family of the xanthophylls.

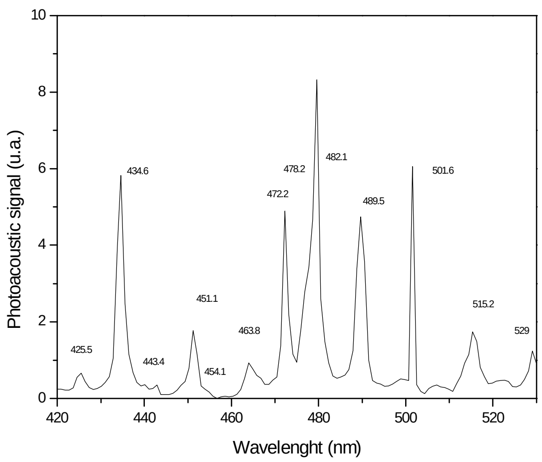

Fig.1- Photoacoustic Visible Spectrum of Carotenoids Concentrate

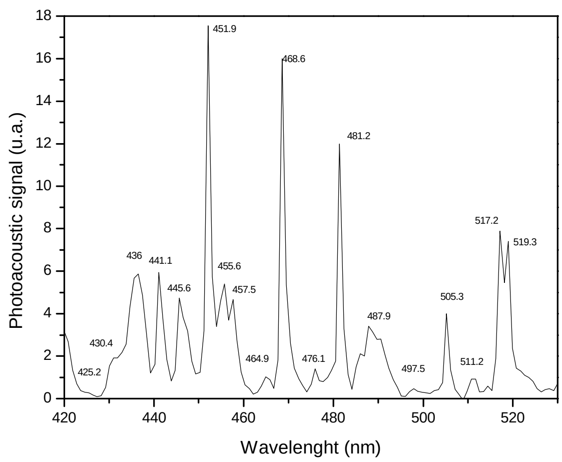

Fig.2- Photoacoustic Visible Spectrum of Carotenoids in Carrot

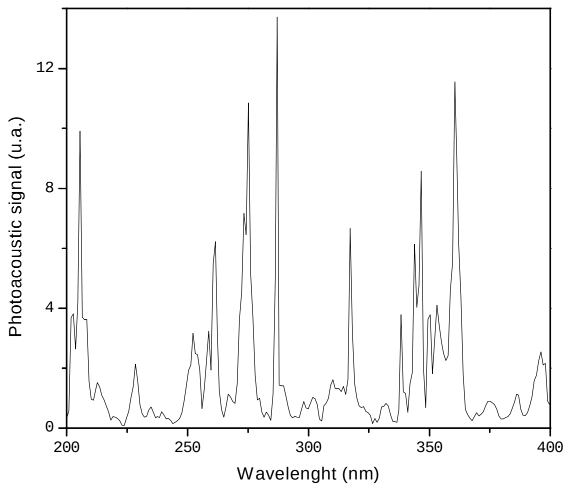

Fig.3- Photoacoustic UV-Spectrum of Flavonoid (quercetin) in mango

Fig.3 shows the observed PAS spectrum of a species of mango in the UV- region. Note the presence of three well resolved bands at (~ 220nm), (250 –280)nm and (330 – 370)nm in good agreement with conventional optical absorption spectrum attributed to the flavonoid type of biomolecules called quercetin.

Table 1: Carotenoids and Flavonoids biomolecules and the investigated biomaterials where they were encountered using PAS technique.

Sample	**Molecules Identified**
Acerola	β-Carotene, Flavonoids
Pumpkin (Abóbora)	β-Carotene, Flavonoids
Watercress (Agrião)	β-Carotene, Flavonoids, Chlorophyll
Lettuce (Alface)	β-Carotene, Flavonoids, Chlorophyll
Beetroot (Beterraba)	β-Carotene
Broccoli (Brocolis)	β-Carotene, Flavonoids
Cabbage (Couve)	β-Carotene, Flavonoids
Cauliflower (Couve-flôr)	β-Carotene, Flavonoids
Spinach (Espinafre)	β-Carotene, Flavonoids
Purple-Cabbage (Repolho Roxo)	β-Carotene, Flavonoids
Tomato (Tomate)	β-Carotene, Lycopene
Orange (Laranja)	β-Carotene, Flavonoids
Tangerine (Tangerina)	β-Carotene, Flavonoids
Carrot (Cenoura)	β-Carotene, Lycopene, Lutein, Lutein 5,6 epoxide
Maize leave (Folha de Milho)	β-Carotene, Chlorophyll, Zeaxanthin
Mango (Manga)	β-Carotene, Flavonoids
Rúcula	β-Carotene, Flavonoids
Cuité (Uva)	β-Carotene, Flavonoids
Pitanga	β-Carotene
Red papper	β-Carotene, Lycopene, and possible capsanthin
Yellow papper	β-Carotene, Lycopene (low concentration)

Conclusions

We have demonstrated that photoacoustic spectroscopy works as a powerful tool in analysis and identification of several carotenoid configurations flavonoid biomolecules in situ and in a non-contact and non-destructive way. Our results in a number of fruits, leaves and vegetables evidence the PAS as a rapid, direct and efficient analytical method in biomaterials particularly in the promising field of photochemistry and photobiology. The well resolved PAS spectra obtained for carotenoids and flavonoids yields the identification of different configurations even in cases of very similar species. Finally, PAS technique can contribute to select and classify fruits, leaves and other vegetables according with their phitotherapeutic and nutritive properties.

Acknowledgements
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References

[1] A. Rosencwaig, Anal. Chemistry, **47** (6) (1975), 592A-604A.
[2] F.A. McDonald, J. Appl. Phys. **49** (1978), 2313-2318.
[3] A. Rosencwaig and A. Gersho, J. Appl. Phys. **47**(1) (1976), 64-69.