Final Scientific Report (2011-2014)

Introduction

PA spectroscopy is an indirect technique in that an effect of absorption is measured rather than absorption itself. Hence the name of photoacoustic: light absorption is detected through its accompanying acoustic effect. The advantage of PA is that the absorption of light is measured on a zero background; this is in contrast with direct absorption techniques, where a decrease of the source light intensity has to be observed. The spectral dependence of absorption makes it possible to determine the nature of the trace components. The PA method is primarily a calorimetric technique, which measures the precise number of absorbent molecules by simply measuring the amplitude of an acoustic signal. In LPAS the nonradiative relaxation which generates heat is of primary importance. In the IR spectral region, nonradiative relaxation is much faster than radiative decay.

The PA effect in gases can be divided into five main steps (Dumitras et al., 2007a, Dumitras et al., 2012a, Dumitras et al., 2012b):

- Modulation of the laser radiation (either in amplitude or frequency) at a wavelength that overlaps with a spectral feature of the target species; an electrooptical modulation device may also be employed, or the laser beam is modulated directly by modulation of its power supply; the extremely narrowband emission of the laser allows the specific excitation of molecular states; the laser power should be modulated with a frequency in the range $^{2}_{th} >> 1/f >> {}^{2}_{nr}$, where $^{2}_{th}$ is the thermal relaxation time, and $^{2}_{nr}$ the nonradiative lifetime of the excited energy state of the molecule.
- Excitation of a fraction of the ground-state molecular population of the target molecule by absorption of the incident laser radiation that is stored as vibrational-rotational energy; the amount of energy absorbed from the laser beam depends on the absorption coefficient, which is a function of pressure.
- Energy exchange processes between vibrational levels (V-V: vibration to vibration transfer) and from vibrational states to rotational and translational degrees of freedom (V-R, T transfer); the energy which is

absorbed by a vibrational-rotational transition is almost completely converted to the kinetic energy of the gas molecules by collisional de-excitation of the excited state; the efficiency of this conversion from deposited to translational energy depends on the pressure and internal energy level structure of the molecule; vibrational relaxation is usually so fast that it does not limit the sensitivity; however, notable anomalies occur in the case of diatomic molecules, such as CO, where vibrational relaxation is slow in the absence of a suitable collision partner, and of the dilute mixtures of CO_2 in N_2, where the vibrational energy is trapped in slowly relaxing vibrational states of N_2; the kinetic energy is then converted into periodic local heating at the modulation frequency.

- Expansion and contraction of the gas in a closed volume that give rise to pressure variation which is an acoustic wave; the input of photon energy with correct timing leads to the formation of a standing acoustic wave in the resonator.
- Monitoring the resulting acoustic waves with a microphone; the efficiency at which sound is transmitted to the microphone depends on the geometry of the cell and the thermodynamic properties of the buffer gas.

From kinetic gas theory it can be estimated that a molecule performs 10^9-10^{10} collisions per second at 1 bar pressure. This means that at atmospheric pressure the photon energy is transformed into an acoustical signal in about 10^{-5}-10^{-6} s. For most polyatomic molecules signal production is even faster. The time needed by the pressure wave to travel from the laser beam area to the microphone in the acoustic cell is therefore in most cases longer than the vibrational relaxation time. For a distance of a few centimeters this transit time is about 10^{-4} s. The time delay between excitation and detection of the pressure wave, however, is influenced not only by energy transfer processes and the transit time, but also by the response time of the gas-microphone system, being about 10^{-4} s or longer (Hess, 1983).

The block diagram of the laser photoacoustic spectrometer for gas studies, is shown in Fig. 1.

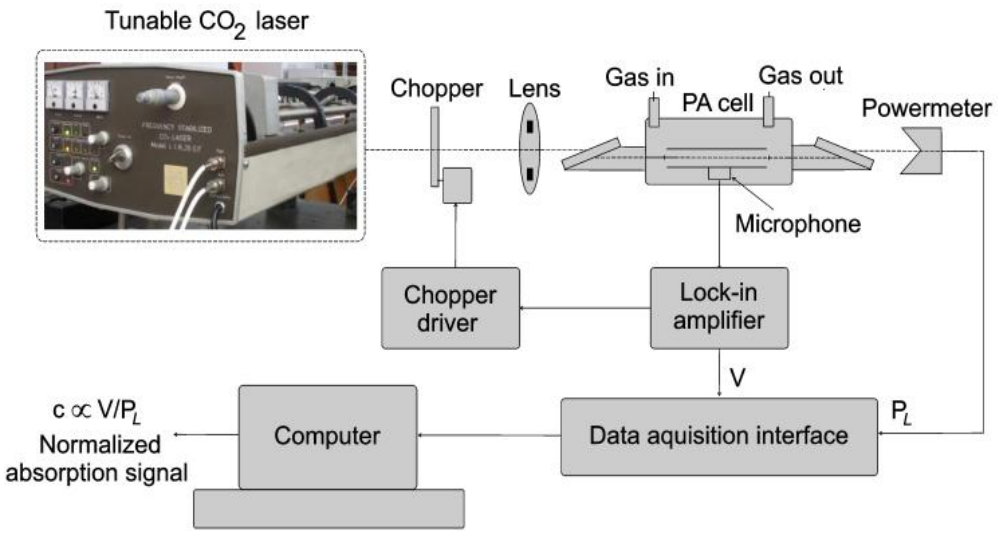


Figure 1. Block diagram of laser photoacoustic spectrometer.

The continuous wave, tunable CO_2-laser beam is chopped, focused by a ZnSe lens, and introduced in the PA cell. After passing through the PA cell, the power of the laser beam is measured by a laser radiometer Rk-5700 from Laser Probe Inc. with a measuring head RkT-30. Its digital output is introduced in the data acquisition interface module together with the output from the lock-in amplifier. All experimental data are processed and stored by a computer (Dumitras et al., 2007a).

An advantage of PA spectroscopy as a tool for trace gas analysis is that very few photons are absorbed as the laser beam passes through the sample cell. As a result, notwithstanding the losses from absorption in the windows, the transmitted beam typically has sufficient power for analyzing samples in successive cells, via a multiplexing arrangement. A multiplexed PA sensor can be used to monitor many different samples simultaneously so that one instrument can be deployed to monitor up to 20 different locations within a clean room, industrial plant or other facility (Pushkarsky et al., 2002).

Following the terminology introduced by Miklos et al. (Miklos et al., 2001), the name "PA resonator" will be used for the cavity in which the resonant amplification of the PA signal takes place. The term PA cell (or PA detector; both terms are used in the literature to describe the device in which the PA signal is generated and monitored) is reserved for the entire acoustic unit, including the resonator, acoustic baffles and filters, windows, gas inlets and outlets, and microphone(s). Finally, PA instrument

(PA sensor) stands for a complete setup, including the PA cell, light source, gas handling system, and electronics used for signal processing.

It is interesting to mention that the *reverse* PA effect, called "sonoluminiscence", consists in the generation of optical radiation by acoustic waves, while the *inverse* PA effect is the generation of sound due to optical energy being lost from a sample, instead of being deposited in a sample as in the usual PA effect (Tam, 1986).

We use an extracavity arrangement because it has several advantages. In spite of a lower laser power available to excite the absorbing gas in the PA cell, a smaller coherent PA background signal makes it possible to increase the overall sensitivity of the instrument. Also, the dynamic range of the PA method is considerably reduced by intracavity operation. Optical saturation may occur for molecules with high absorption cross section while uncontrollable signal changes may be obtained at higher overall absorption in the PA cell, because the loss of light intensity influences the gain of the laser. This effect may cause erroneous results when the sample concentration changes are large. Therefore, high-sensitivity single-and multipass extracavity PA detectors offer a simpler alternative to intracavity devices.

1: Analysis of the exhaled ethylene from patients treated by anti-tumour radiotherapy

The first objective was to measure the exhaled ethylene from patients receiving radiation treatment and to compare the results with healthy subjects in order to correlate the ethylene concentrations with the level of oxidative stress.

Radiation therapy uses ionizing radiation (e.g. X-rays) to kill cancer cells and shrink tumours. When considering ionizing radiations, a substantial part of the total interactions concerns water molecules, water being the major component of living tissue present in all biological systems. Consequently, water ions and radicals are mainly generated inside tissues as primary reactive species. Those reactive species (free radicals) interact with biomolecules and damage them (indirect effect of radiation); in particular, they can start lipid peroxidation events on cell membranes [19-21].

We analyzed the exhaled breath air from cancer patients, subjected to radiation treatment, based on X-ray external beam, after tumor surgery.

To analyze the bag contents, firstly we evacuated thoroughly the previous gas mixture from all the handling system, including the PA cell, traps, pipes etc., and then we flushed the system with pure nitrogen at atmospheric pressure for 10-15 minutes. After a second vacuum cleaning, the exhaled air samples were transferred in the PA cell and analyzed.

Fig. 2 presents the levels of ethylene experimentally measured for a healthy subject (female, 28 years old) and three patients (females, aged 32, 53, 77 years) with mammary cancer treated by X-ray radiation with a dose of 8 Gy. The concentration level of ethylene for the first patient before and immediately after the X-ray therapy shows an increase from ~ 0.018 ppm to ~ 0.023 ppm, for the second patient the concentration level of ethylene before X-ray therapy was ~ 0.021 ppm and immediately after the X-ray therapy ~ 0.03 ppm, for the third patient the concentration level of ethylene before and after the X-ray therapy was ~ 0.017 ppm and ~ 0.023 ppm, whereas for a healthy subject they are ~ 0.006 ppm. After X-ray irradiation we observed that the ethylene concentration increases, showing that lipid peroxidation took place and it is already possible to detect the process in the very first minute after irradiation.

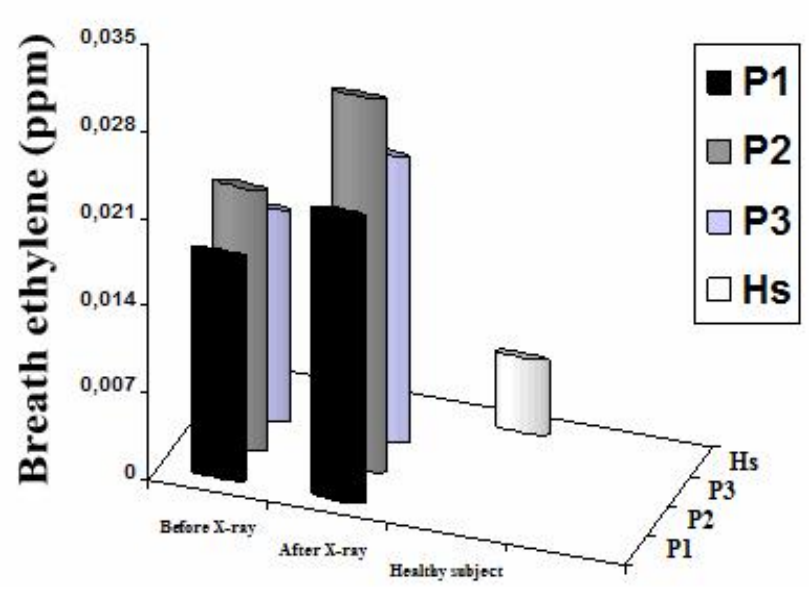


Fig. 2. The level of ethylene for a healthy subject and for three patients treated by anti-tumour radiotherapy.

The interaction of X-rays with the human body modifies the oxidative stress status through an increase in the peroxidation processes initiated by the free water radicals which were generated by the indirect radiation effect in the living tissue. A consistent part of these peroxidation events produces ethylene by lipid peroxidation. The fraction of the produced ethylene present in the exhaled breath reveals us the scale of tissue damage in the body following each X-ray session.

2. Analysis of exhaled ammonia and exhaled ethylene from patients treated by haemodialysis

The second objective of the project is to detect and measure the exhaled ammonia and ethylene from patients with renal failure receiving haemodialysis. The analysis of ethylene and ammonia traces from human breath would provide the necessary insight into severity of oxidative stress and metabolic disturbances and assure optimal therapy and prevention of pathology at patients on continuous haemodialysis.
Human bodies use ammonia in a number of ways, including for the maintenance of the normal pH balance necessary to sustain life. Ammonia is processed in the liver, kidneys and skeletal muscles. Typically, ammonia and ammonium ions (in a healthy individual) are converted into urea in the liver through the urea cycle (Krebs-Henseileit cycle). As small molecules, ammonia and ammonium ions can penetrate the blood-lung barrier, being exhaled with the breath.
In the case of kidney dysfunction, urea is unable to be excreted, causing an excessive build up of ammonia in the blood, inducing the decay of all organism functions and causing morbidity and mortality.
Kidney dialysis is evaluated with a dimensionless parameter called urea reduction ratio (URR) that compares the pre and post dialysis levels of blood urea nitrogen (BUN). This calculation requires taking blood samples and generally sending them to a lab for BUN determination. A surrogate for the URR is breath ammonia reduction ratio (BARR - derived from the measurements of predialysis and during dialysis breath ammonia concentrations). For BUN (URR) under normal circumstances, results are not available in less than 12-48 hours, while for BARR with LPAS the

results are available in less than 10 minutes.

To the normal buildup of urea in the body, a particularly increase in uremia at dialyzed patients should be added due to the oxidative stress. The oxidative stress is a persistent manifestation at patients with renal failure, where the loss of balance between free radical or reactive oxygen species (ROS) production and antioxidant systems is more pregnant, with strong negative effects on carbohydrates, lipids and proteins. In order to make the distinction between the level of uremia due to the normal physiological processes in the body and those induced by the stress of undergoing the dialysis, the parallel determination of ethylene concentration is required.

Ammonia biomarker was measured using the LPAS system and subjects were recruited from patients receiving dialysis treatment at the renal dialysis clinics at the IHS Fundeni, Bucharest.

Experimental determinations in order to detect traces of ammonia and also to measure the urea level were performed for a healthy volunteer and for 13 patients with kidney failure (Popa et al., 2011b, Popa et al., 2013c).

Analysis of pre-dialysis urea level and post-dialysis urea level were made at MedCenter, Bucharest and the results are presented in Fig. 3.

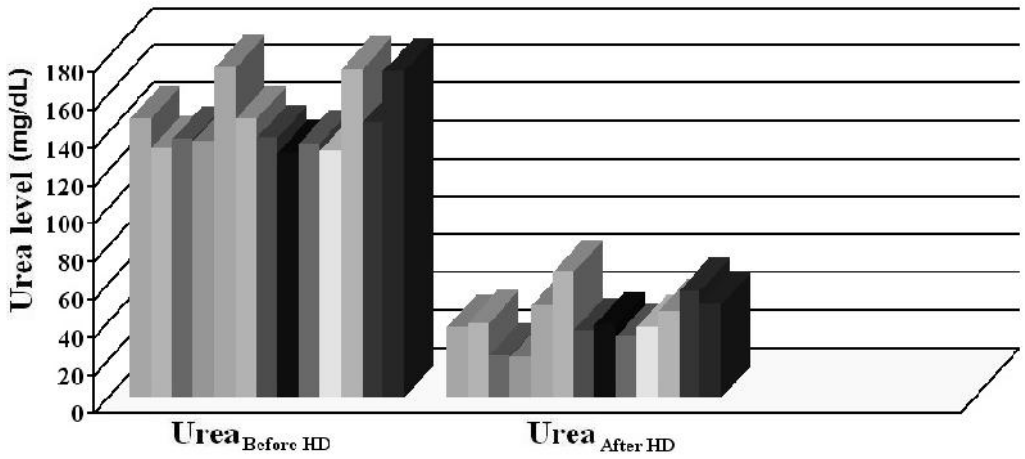


Figure 3 Urea data measured for 13 patients with kidney failure.

The exhaled air samples were collected before, and after the dialysis procedure stoped. We have analyzed ammonia exhaled from patients receiving HD for treatment of kidney failure.

Experimental measurements of breath ammonia concentrations for the patients with renal failure and for the healthy subject were performed and the results are presented in Fig. 4. The control value for breath ammonia was 0.25 ppm.

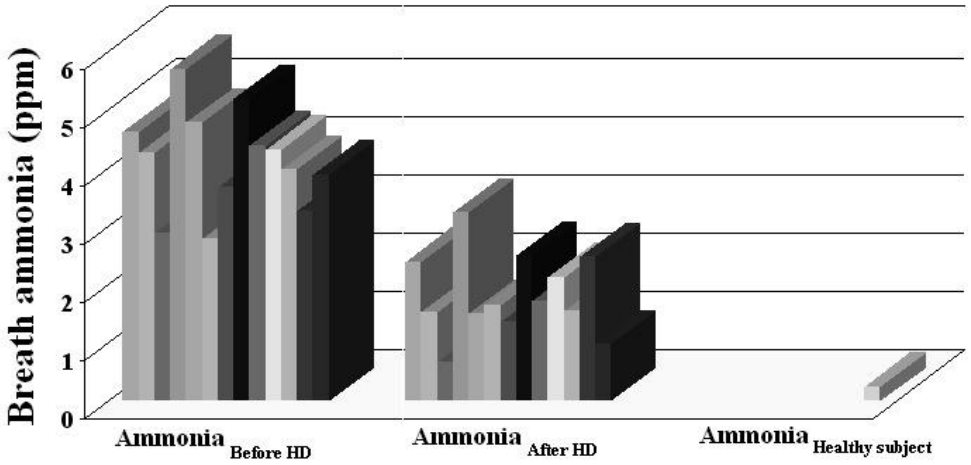


Figure 4. Ammonia concentration for 13 patients with kidney failure.

In Fig. 4, we observed a reduction of ammonia concentration in exhaled breath at patients under HD treatment, which means that ammonia detection in human breath using LPAS system can be used for determining the exact time necessary for the desired state of HD for a patient with kidney failure at every session and, in the same time, could serve as a broad noninvasive screen for incipient kidney disease.

We can see also a remarkable positive correlation between urea data from Fig. 3 and the breath ammonia concentration from Fig. 4.

The ammonia test is noninvasive, easily repeated, and does not have the discomfort or embarrassment associate with blood and urine tests.

These measurements (Popa et al., 2011b, Popa et al., 2013c, Popa et al., 2013b) were possible because of the high sensitivity of our CO_2 LPAS system, sensitivity that was obtained through successively improvements in optics, laser source and electronics (faster response, low noise equipment).

In the recent years there is a large increase in the areas related to the developments in prevention of diseases, especially in explaining the role of oxidative stress. The lipid peroxidation (LP) and oxidative stress contributes to morbidity at HD patients. So, it will be relevant to analyze the impact of oxidative stress and its related species (ethylene) immediately after dialysis treatment in order to prevent trauma in renal failure of elderly patients (Popa et al., 2013c, Popa et al., 2013b).

First I tested the ability of the LPAS system to distinguish the subjects assumed to be healthy. In this way, 10 age matched healthy subjects (Hs) served as controls. The control volunteers (considered healthy) were non-smokers, non alcoholic and non diabetic, without kidney and lung diseases, or other known chronical affections. It should be pointed out that the Hs did not receive HD treatment.

The Hs were advised to use antiseptic mouthwash (the same condition applies for the HD patients) before breath sampling, to minimize the risk of contamination issues and to avoid the oral bacteria.

The average concentration of breath ethylene at Hs was 0.0063 ppm (see Fig. 5) with a normal exhalation flow rate.

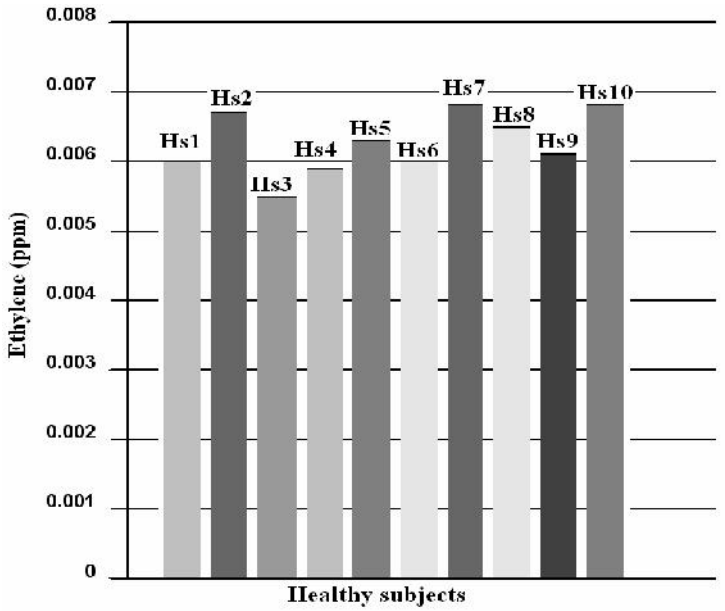


Figure 5. Breath ethylene for 10 healthy subjects – Hs.

While much is known about the effect of renal disease and dialysis on the composition of blood, little is known about their impact on the composition of breath. This analysis focuses on the ethylene changes in the exhaled breath composition of elderly HD patients immediately after the treatment of renal disease. Five patients with renal failure (age between 70 and 80 years old) from IHS Fundeni, Bucharest, participated twice in this study, which was designed to explore the effect of urea (from the blood) and the HD treatment on the composition of exhaled breath. Patients had been on HD for periods ranging from 7 to 8 years and were non-smokers, non

diabetic and had not consumed alcohol for at least 10 hours prior to their arrival at the clinic. Over a four hour HD procedure (typically per week), breath ethylene was collected from participants before and immediately after the procedure.

For this study the subjects were asked to exhale into sample bags at a normal exhalation flow rate (the same exhalation technique like Hs).

Table 1 summarizes the characteristic parameters for ethylene breath test protocol (Popa et al., 2013c, Popa et al., 2013b).

Table 1. Essential parameters of ethylene breath test protocol

LPAS system parameters	**Specifications**
PA cell total volume	≈1000 mL
Breath sample flow rate	600 sccm
Working temperature	23 - 25^0 C
Breath sample time collection	≈ 10 s
Breath sample time transfer	≈ 2 minutes
Breath sample time analysis	≈ 3 minutes

Figures 6 and 7 show the experimental results of breath ethylene and urea level for the participants with renal failure before and after HD procedure.

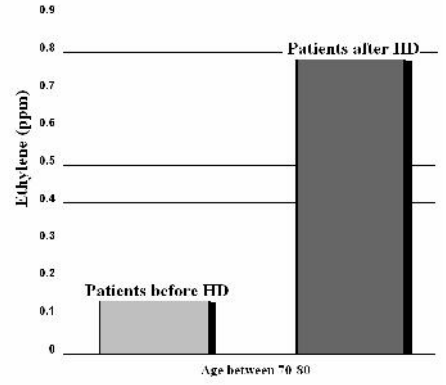
All measurements were made at 10.53 μm CO_2 laser line/10P (14), where the ethylene absorption coefficient has the largest value (30.4 $cm^{-1}atm^{-1}$ at 949.479 cm^{-1}).

Exhaled breath ethylene concentrations in renal failure at elderly patients are considerably changed immediately after the treatment (see figure 6), suggesting that subjects are under oxidative stress during HD, and ethylene may be considered a suitable biomarker for LP and oxidative stress in this case.

Because renal failure arises from the inability of the kidneys effectively to work and to clear the blood, an accumulation of urea in the blood is produced; HD treatment helps the patient to remove more urea from the blood. There is a reduction in the urea concentration in the blood of patients as HD proceeds. The results are given in Fig. 7.

We have monitored the evolution of the oxidative stress during HD treatment using the exhaled ethylene as a biomarker. We can observe that in elderly patients with renal failure, and particularly in those submitted to HD treatment, the ethylene concentration is increased, proving the existence of reactive oxygen species.

The ethylene concentration in breath is unlikely to be due to a reservoir of ethylene stored in tissue, since ethylene is highly volatile, not significantly metabolized by the body and not soluble in body fat. So, ethylene rapidly diffuses into the bloodstream after generation, being transported to the lungs to be excreted (in the expired breath), from where can be collected (Popa et al., 2013b).



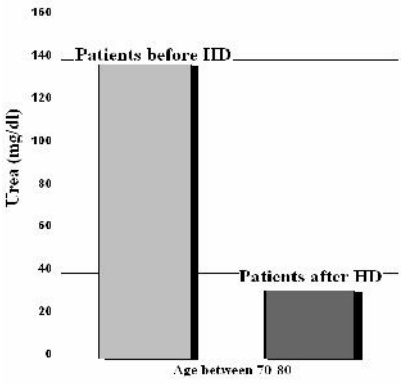


Figure 6 The average levels of ethylene - 5 subjects. **Figure 7** The average levels of urea - 5 subjects.

HD may aggravate and intensify the oxidative stress. This seems to be due to multiple factors including an increase in the production of agents from oxidative metabolism, and a decrease in anti-oxidant defenses. Other factors such as the use of low biocompatible membranes and purity of dialysis water, chronic inflammatory state, diabetes, anemia, hypertension, etc., are linked to an increase in LP. Also age, lifestyle and underlying condition affect the overall oxidative stress status of HD patients.

HD is a method for extracorporeal removing of the waste products such as creatinine and urea, as well as water from the blood when the kidneys are in kidney failure. HD is one of three renal replacement therapies (the other two being renal transplant and peritoneal dialysis).

HD was accomplished with BAXTER dialysis machines using DICEA (and XENIUM) high performance cellulose diacetate hollow fibre dialyser-gamma series (DICEA 170G) with following characteristics: surface area of 1.7 m^2, ultrafiltration rate 12.5 ml/hr/mmHg, inner diameter of 200 microns and membrane thickness of 15 microns. Experimental measurements in order to detect traces of ethylene and ammonia were performed for a healthy volunteer (C. A. male, 26 years old) and for 6 patients with renal failure.

Participants were recruited from patients receiving HD treatment at the renal dialysis clinics and were dialyzed 3 times per week, with a 4 h dialysis session, instructed to use antiseptic mouthwash before each breath sampling, to avoid oral bacteria.

This time the exhaled air samples were collected before, during (about 1 hour after the start of HD) and immediately after the HD procedure. Experimental measurements of breath ethylene and ammonia concentrations for the patients (P1-P6) with renal failure and for the healthy subject (P0) were performed and the results are presented in Figures 3 and 4, respectively. The control P0 values are 0.006 ppm ethylene and 0.25 ppm ammonia. The details for patients P1 to P6 are introduced in Table 4.2. All measurements were made at 10P(14) CO_2 laser line (10.53 μm), where the ethylene absorption coefficient has the largest value (30.4 $cm^{-1}atm^{-1}$), and at 9R(30) CO_2 laser line (9.22 μm), where the ammonia absorption coefficient has the maximum value of 57 $cm^{-1}atm^{-1}$.

Particular data of patients are summarized in Table .2 (Popa et al., 2011b, Popa et al., 2013c).

Table 2. The particular data of patients and the experimental measurements of breath ethylene and ammonia concentrations (± 10 % data error)

Patients	**Gender**	**Age**	**HD since**	**U_{preHD} (mg/dl)**	**U_{postHD} (mg/dl)**	**C_2H_4 (ppm)**			**NH_3 (ppm)**		
						before HD	during HD	after HD	before HD	during HD	after HD
P1	Male	67	2005	147	37	0.03	0.13	0.52	4.63	3.58	2.39
P2	Male	80	2004	131	39	0.23	0.51	0.93	4.28	2.82	1.53
P3	Male	79	2008	136	22	0.17	0.31	0.91	2.89	2.06	0.67
P4	Male	22	2010	135	21	0.14	0.19	0.84	5.71	4.08	3.24
P5	Male	54	2010	174	48	0.18	0.43	0.89	4.79	3.07	1.5
P6	Male	66	2005	147	66	-	-	-	2.8	2.01	1.66

A special mention should be made: NH_3 is a highly adsorbing compound and the results of successive measurements are often altered by the molecules previously adsorbed on the pathway and cell walls. To ensure the quality of each measurement,

an intensive cycle of N_2 washing was performed between samples, in order to have a maximum increase of 10% for the background photoacoustic signal. It has to be underlined that the measured photoacoustic signal is due mainly to the absorption of ammonia and ethylene, respectively, but some traces of CO_2, H_2O, ethanol, etc., influence the measurements (overall contribution is less than 10%).

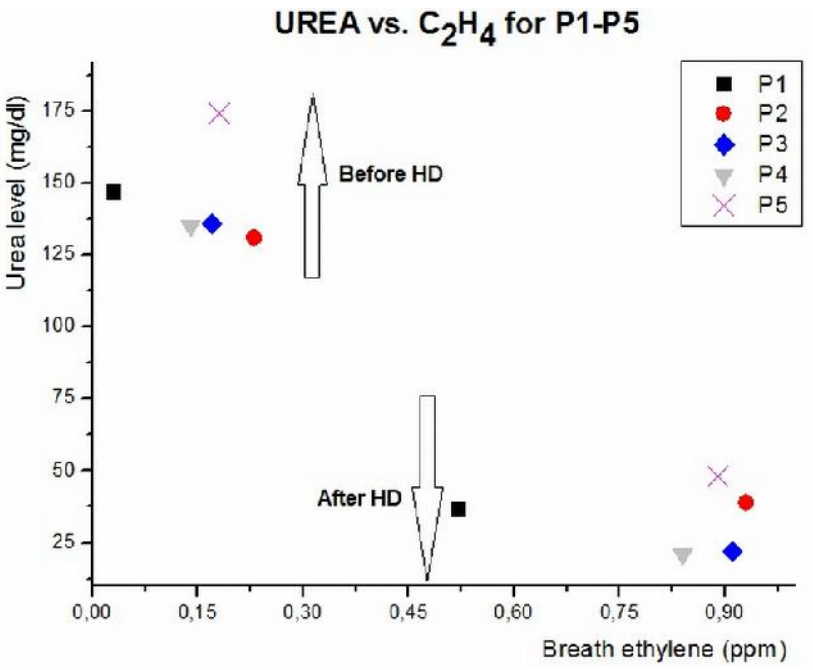


Figure 8. Breath ethylene concentration measured for 5 patients with renal failure and correlation with Urea level.

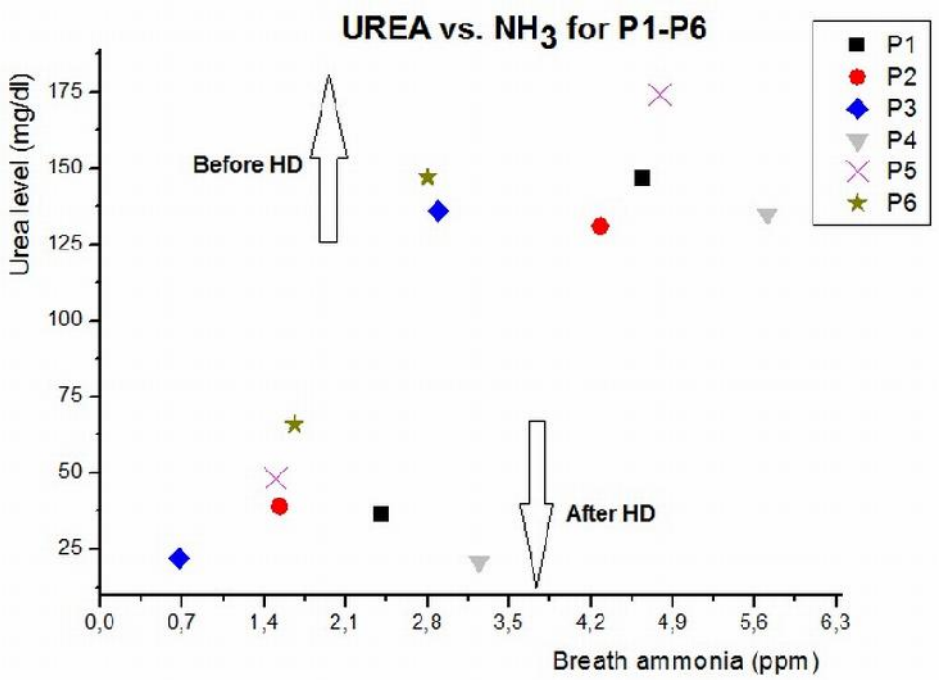


Figure 9 Breath ammonia concentration measured for 6 patients with renal failure and correlation with Urea level.

As expected, we see that, (Fig. 8), immediately after HD treatment, the ethylene concentration increases, proving the presence LP and showing an imbalance between oxidant and antioxidant systems.

Another biomarker present in patients on HD is ethane which shows absorption at ~ 3.4 μm (lead-salt diode laser) and was analysed by Patterson et al. (Patterson et al., 2007) Stevenson et al. (Stevenson et al., 2008) and Handelman et al. (Handelman et al., 2003) in exhaled air during HD treatment. They observed a significant peak in oxidant stress levels and demonstrated endogenous production of ethane by the patient whilst on HD.

HD is associated with increased oxidative stress and all treated patients are exposed to this stress. This observation appears to be due to an increased production of free radicals during HD and immediately after HD and a net reduction of many antioxidants. In order to verify this hypothesis also further studies are required.

In Fig. 9, as expected, we observed, a reduction of ammonia concentration in exhaled breath at patients under HD treatment, which means that ammonia detection in human breath using LPAS system can be used for determining the exact time necessary for the desired state of HD for a patient with end stage renal disease at every session and,

in the same time, could serve as a broad noninvasive screen for incipient renal disease (Popa et al., 2011b).

The most important result is the correlation found between Urea data (measured by blood analysis) and the individual breath ammonia and ethylene concentrations (measured by photoacoustic technique), shown in Figs. 8 and 9 for another six patients.

We have found out that the composition of exhaled breath in patients with renal failure contains not only ethylene, but also ammonia and gives valuable information for determining efficacy and endpoint of HD.

Analysis of ethylene and ammonia traces from the human breath may provide insight into severity of oxidative stress and metabolic disturbances and may assure optimal therapy and prevention of pathology at patients on continuous HD.

As future work, we recommend the increasing of antioxidant intake level in HD patients and then compare the oxidative stress production, keeping the present results as reference and the exhaled ammonia/ethylene as specific biomarkers (Popa et al., 2011b).

3. Analysis of exhaled ethylene from smokers using traditional or electronic cigarettes (smoke vs. vapor emission)

As a third objective we plan to compare the ethylene concentrations at subjects who inhale cigarette smoke with subjects who inhale electronic smoke, and passive smokers.

The CO_2 LPAS is suitable for the detection of ethylene in exhaled breath (Popa et al., 2014a, Popa C., 2014, Popa et al., 2014c, Popa et al., 2014b), producing feasible and reproducible results which discriminate active smoking with E-cigarettes (electronic) *vs.* T-cigarettes (traditional).

The E-cigarette closely imitates T-smoking since it tastes, looks and also feels like a traditional one. When “vaping” the E-cigarette inhaling produces both the tactile and craving satisfaction which T-cigarettes seeks and generates a vaporizing process that releases a vapor mist that evaporates into the air within just a few seconds.

Since the introduction of this product to the consumer marketplace, a number of new companies around the world have started producing them in order to take advantage of the overwhelming positive response being generated by the consumer (Popa 2014a, Popa 2014b).

While we can't make the claim that E-cigarettes are healthier, we can point out how T-cigarettes are harmful to our health and can put us at higher risk of a whole host of conditions, including: stroke, heart attack, lung cancer, throat cancer, pneumonia, osteoporosis, Alzheimer's and countless others.

This chapter section reports the LPAS as a sensitive, real time and non-invasive tool to monitor at different time intervals the concentration of ethylene at E-cigarettes smokers and T-cigarettes smokers (Popa 2014a, Popa 2014b).

The data analysis, were conducted for 5 days with ten male's smoker subjects (five of them: smokers only of E-cigarettes and the other five: smokers only of T-cigarettes).

To evaluate the breath ethylene we choose to analyze the effect of the inhalation with E-cigarettes (with 0.5 mg nicotine/drop, 10 mg of ***nicotine/20 drops***) and T-cigarettes (with 0.5 mg /cigarette, 10 mg of ***nicotine***/pack: 0.5 mg x 20 cigarettes) at different time intervals (at 9^{00} a.m. and 10^{00} a.m.) in two sessions.

The subjects were not in the stage of smoking cessation attempt, were non-alcoholic and non-diabetic, without any chronic mental or physical health problem. Also the ten male's smoker subjects were asked, to avoid coffee and alcohol for at least 6 hours prior to their participation in the study and provided three breath samples every day between 8^{30} a.m. and 10^{00} a.m. (at 8^{30} a.m. collection of breath sample before smoking, at 9^{00} a.m. collection of breath sample after the first cigarette and at 10^{00} collection of breath sample after the second cigarette inhalation) over a period of 5 days.

The T-cigarette smoker smoked one cigarette/session/0.5mg nicotine at 9^{00} a.m., with 15-20 puffs/cigarette and 10-15 seconds interpuff interval, during ≈ 10 min smoking session. After that, with a break of about 50 min, the subject start to smoke the second T-cigarette/second in the session (used similarly conditions to the first cigarettes).

In the same time the E-cigarette smoker (similarly to T-cigarette smoker) put one drop with 0.5 mg of nicotine E-liquid in the atomizer and start to inhale with 15-20 puffs/drop, 10-15 second's interpuff interval and ≈ 10 min "vaping" session (see in the fig 10). After a break of about 50 min, each smoker repeated the entire session with one E/T cigarette one more times.

The volunteers were asked to smoke the same brand of cigarette to avoid variability in smoke composition (it is known that cigarette from different brands can generate different ethylene levels). Immediately after the final puff of each cigarette, the

smoker exhaled in the sample bag through the mouth. All the volunteers used the same procedure for inhalation of smoke/vapours by cigarettes.

All the information's published about the volunteers was the subject to their permission and are provided in Table 3 (Popa 2014a, Popa 2014b).

Table 3 Subjects information's for T-cigarette smoke and E-cigarette vapours exposure

Subject	**Gender**	**Age**	**Subjects height (m)**	**Subjects weight (kg)**	**Smoker since**
S1	Male	23	1.81	73.0	2011 (E-cig.)
S2	Male	28	1.83	97.0	2010 (E-cig.)
S3	Male	31	1.62	56.0	2009 (E-cig.)
S4	Male	29	1.79	78.0	2011 (E-cig.)
S5	Male	23	1.68	83.0	2011 (E-cig.)
S6	Male	35	1.78	98.0	2011 (T-cig.)
S7	Male	32	1.78	81.0	2008 (T-cig.)
S8	Male	37	1.93	99.0	2007 (T-cig.)
S9	Male	27	1.65	62.0	2009 (T-cig.)
S10	Male	28	1.84	73.0	2009 (T-cig.)

Figure 10 shows the average concentrations of breath ethylene for five subjects, before and after exposure to one E-cigarette/session.

Each breath smoker was investigated for 5 days with 2 exposure session/day, one cigarette/session and about 50 min break between sessions.

The baseline for E-smokers was: 20 ppb (the breath sample was collected before the exposure to E-cigarette: at 8.30 a.m.), and after the first E-cigarette inhalation/Session 1, the mean ethylene level for Subject 1 (S1) was about 47 ppb, for S2: 45 ppb, for S3: 47 ppb, for S4: 53 ppb while for S5 in Session 1: 49 ppb.

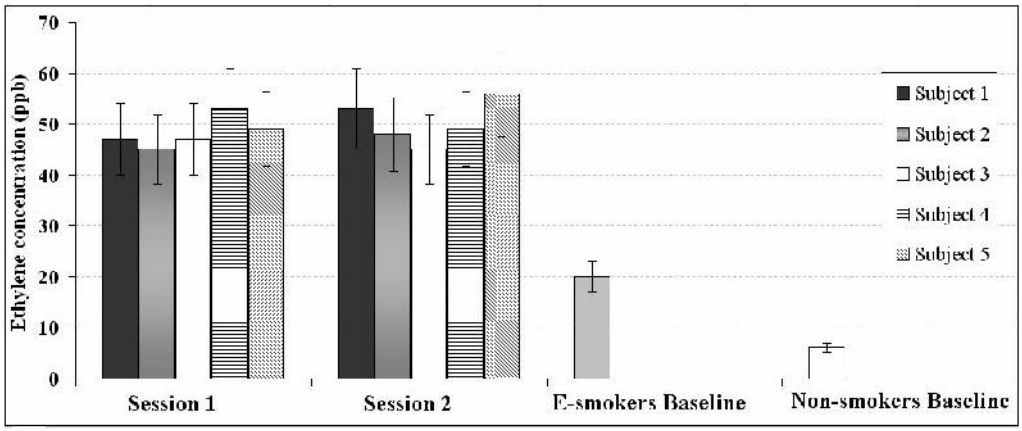


Figure 10 Breath ethylene average levels for five E-cigarettes smoker volunteers

For the session 2 the values of ethylene concentrations for the exhaled breath samples were: S1-53 ppb, S2-48 ppb, S3-45 ppb, S4-49 ppb whereas for S5 the value was: 56 ppb.

Figure 11 shows the average concentrations of breath ethylene for five T-cigarettes smokers, before and after exposure to T-cigarettes.

The baseline for T-smokers was: 27 ppb (before the exposure to T-cigarette at 8.30 a.m.), and immediately after the first T-cigarette inhalation (Session 1), the mean ethylene level increased for S1 at 145 ppb, following that after the second T-cigarette inhalation (Session2) the mean ethylene concentration to increased more at 187 ppb.

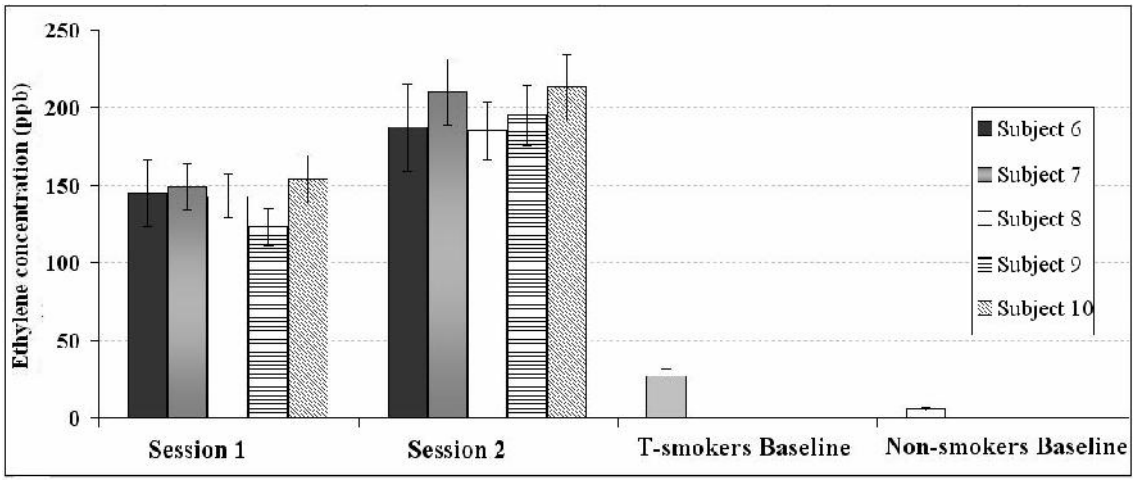


Figure 11. Breath ethylene average levels for five T-cigarettes smokers.

For the other breath samples in Session 1 and Session 2 exhaled ethylene breath were increased at 149 ppb and 210 ppb for S2, 143 ppb and 185 ppb for S3, 123 ppb and 195 ppb for S4, and for S5 the values are: 154 ppb and 213 ppb.

The results were also compared to the ethylene concentration of a non-smoker subject (6 ppb).

It should be pointed out that the E-cigarettes volunteers did not receive T-cigarettes and the T-cigarettes volunteers did not receive E-cigarettes.

Reactive gases like in the smoke can cause damage and breath ethylene can be a response from the damage of the human lung tissue .

Based on literature data (Eissenberg, 2010, Gao, 2014, Vansickel, 2010, Bullen, 2010) and compared with our results, we hypothesized that E-cigarettes are safer than T-***cigarettes*** because the ethylene concentration from breath of E-smokers was found to be smaller at different time intervals (9^{00} and 10^{00} a.m.).

In the present study, both the feasibility and the importance of monitoring exhaled ethylene from different subjects have been shown. The ethylene gas, a biomarker of oxidative stress, has been measured using a CO_2 laser based photoacoustic spectrometer.

The results obtained here give the useful information that smoking T-cigarettes, which release in tobacco smoke a complex chemical mixture of combustion compounds (like burned nicotine and tar), causes adverse health outcomes, particularly cancer, cardiovascular and pulmonary diseases, through mechanisms that include DNA damage, inflammation, and oxidative stress.

Oxidative stress from exposure to tobacco smoke has a role in the pathogenic process leading to chronic obstructive pulmonary disease. The evidence on the mechanisms (lipid peroxidation) by which T-smoking causes disease indicates that there is no risk free level of exposure to tobacco smoke.

E-cigarettes (where nicotine is released into vapors) may help reduce **smokers'** exposure to toxins. Nicotine (while is a **highly addictive** substance), is not what causes cancer for smokers or for the people around them who breath their second hand smoke.

In summary, the study revealed that E-cigarettes are not so dangereous to cause cancer at smokers, because the ethylene in this case was found to be in smaller

concentrations. That is why E-cigarettes may provide an alternative or a substitute to T-cigarettes smoking.

The measurements presented here confirms that the analysis of smokers exhaled breath with CO_2 laser-photoacoustic spectroscopy based instruments is a reliable and non-invasive method, with potential in monitoring the ethylene biomarker from active smoking breath samples.

4. Analysis of ethylene in organic fruits and vegetables versus non-organic fruits and vegetables

The fourth objective shall compare the ethylene concentrations from organic and non-organic food (fruits and vegetables) considering the last category as undergoing stress conditions.

The term "organic" refers to the way farmers grow and process agricultural products, such as fruits and vegetables. For organic food, farmers don't use artificial hormones or irradiation on their plants, pesticides and herbicides are restricted, the routine use of drugs, antibiotics and wormers are disallowed, genetically modified crops and ingredients are banned.

For non-organic food, farmers apply synthetic pesticides and herbicides to combat insects and weeds, hormones, fertilizers and antibiotics (this is especially dangerous for humans when we ingest, because we encourage the rapid spread of antibiotic-resistant infections), irradiation and contaminated sewage sludge (it was showed that large amounts of this may contribute to chronic illnesses). In the case of the final product artificial colors or preservatives may be also added.

Stress is the "disease of the century". At plants, stress is produced by irradiation, exposure to high temperature, flooding, drought, freezing, growth hormones, antibiotics, pesticides and herbicides (toxicity). These metabolic disturbances in fruits (vegetables) are followed by significant and rapid changes in the rate of ethylene emission. Irradiation (one of the metabolic disorder) preserves the food by disrupting the biological processes that lead to decay of food quality. Radiation interacts with water and other biological molecules in a food system and produces LP, which generally act as oxidizing agents and can cause several changes in the molecular structure of organic matter. Since this gas is also active within humans, it can lead to uncontrolled cell division or cancer.

To test the quality of non-organic bananas, we analyzed the level of ethylene by using the LPAS method, beeing one of the most used approaches for sensitive, good selective and real time monitoring trace gas detection. LPAS can routinely detect trace gases quantities down to 1 ppb (parts per billion).

The following important parameters were used throughout the experiments for the detection of ethylene gas at bananas:

- Non-organic bananas cuvette pressure: ≈ 1024 mbar;
- Responsitivity of the PA cell: 433 cmV/W;
- Synthetic air: Linde Gaz Romania, 20% oxygen and 80% nitrogen (impurities: hydrocarbons max. 0.1 ppmV, nitrogen oxides max. 0.1 ppmV);
- Nitrogen: Linde Gaz Romania, nitrogen 5.0 (purity 99.999%) and 6.0 (purity 99.9999%);
- Working CO_2 laser line: 10P(14), where we have a maximum absorption coefficient for ethylene: λ= 949.479 cm^{-1} , α= 30.4 $cm^{-1}atm^{-1}$;
- Operating temperature: 23 - 25^0C;
- Glass cuvette total volume: ≈1800 mL;
- PA cell total volume: ≈1000 mL;
- Non-organic banans samples analysis time: ≈ 3 minutes.

To increase the accuracy of the measurements for the analysis of ethylene in bananas, we took several supplementary measures, such as glass cuvettes (used to contain non-organic bananas) for preserving the sample gas and a trap filled with potassium hydroxide pellets (KOH) for prevention of possible inhibition of samples by accumulation of CO_2 (Bratu *et al*., 2011).

To analyze the non-organic bananas glass cuvette contents, firstly we evacuated thoroughly the previous gas mixture from the entire handling system, including the PA cell, traps, pipes etc., and then we cleaned the system for few minutes. After a second vacuum cleaning, the gas from the bananas sample was transferred in the PA cell and analyzed.

Regardless of the non-organic bananas glass cuvette chosen, the system of sample collection must be carefully checked to ensure that there is no ethylene loss (leakage, decomposition, adsorption to the sample cuvette), or generation of ethylene as a result of chemical reactions within the cuvette. If the sample cuvettes are to be reused, it is equally important to ensure that the cuvettes do not have an ethylene memory (release

of ethylene adsorbed onto the inner lining into subsequently collected samples). Possible accumulation of ethylene was prevented by washing the glass cuvette with nitrogen (it is a pure clean non expanding inert gas) at atmospheric pressure for few minutes.

The non-organic banans were sorted and evaluated only at four ripening stages and expressed in grams (g): green bananas (between 150 and 160 g), half green/half yellow bananas (between 150 and 170 g), completely yellow bananas (between 100 and 125 g), and yellow with brown flecks bananas (between 115 and 130 g).

The fruits were obtained from international dealers (supermarkets), and transported to the Laboratory for analysis of ethylene hormone using LPAS technique.

All fruits were stored at 4^0C for subsequent use. Before starting the ethylene analysis, all fruits were acclimatized over 1 h at room temperature (23 - 25^0 C) and then introduced into the glass cuvette for measurements.

The fruit samples were flushed with air flow at atmospheric pressure (1024 mbar) and the resulting gas from the glass samples was transferred in the PA cell and analyzed.

Over a 30 minutes period we achieved a real-time measurements of ethylene emission produced by non-organic bananas.

The principal objective of the investigation was to detect the ethylene gas from stage 1, stage 3, stage 6 and stage 7 of bananas fruits in biotic conditions (Fig. 12).

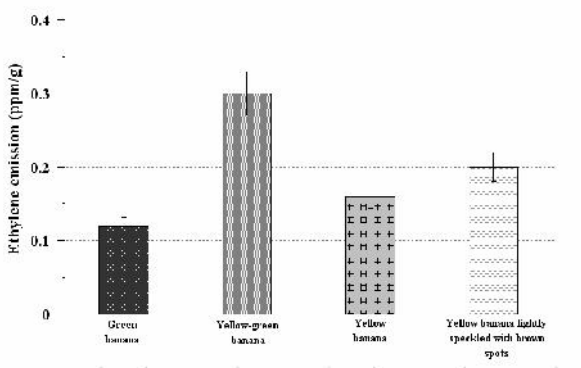


Fig. 12 Ethylene production for different developmental stages at bananas fruits in biotic conditions; measurement errors are ± 10% for all bananas sampled

From Fig. 12 we can observe that the production of ethylene, in stage 1 was 0.12 ppm/g, in stage 3 it was 0.3 ppm/g, in stage 6 it was 0.16 ppm/g and in stage 7 it was 0.2 ppm/g.

In biotic conditions, we found the highest rates of ethylene production at non-organic bananas in half green/half yellow stage.

The second objective of the investigation was to detect the ethylene gas from stage 1, stage 3, stage 6 and stage 7 of bananas fruits in abiotic conditions (Fig. 13).

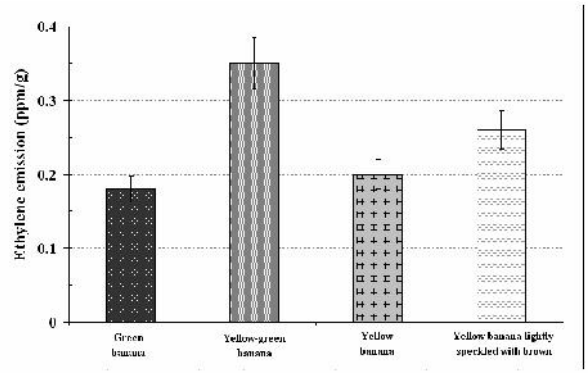


Fig. 13 Ethylene production for different developmental stages at bananas fruits in abiotic conditions; measurement errors are ± 10% for all bananas sampled

In this case we applied to the fruit samples a nitrogen flow at atmospheric pressure and the resulting gas from the glass samples was transferred in the PA cell and analyzed.

The ethylene emission is forced and starts to rise at all four stages.

We can observe that the production of ethylene, in stage 1 rises at 0.18 ppm/g, in stage 3 it rises at 0.35 ppm/g, in stage 6 it rises at 0.2 ppm/g and for stage 7 it rises at 0.26 ppm/g.

Ethylene biosynthesis requires the presence of oxygen, and the replacement of air flow with nitrogen flow may create biochemicals injury and can ultimately lead to the increased production of ethylene.

Nitrogen can be one of the most critical nutrients in non-organic bananas production and can create quality problems promoting excessive development that depresses the fruit.

Since bananas are climacteric fruits, they produce ethylene in ripening process and hasten the metabolic processes (such as sweetened banana). The curing degree of the first green stage banana contains a lot of starch, having a taste similar to potatoes, and a very low concentration of ethylene was found. As a result of enzymatic reactions initiated by ethylene, at stage 2-green with trace of yellow stage, ethylene is converted to soluble sugars, giving sweet taste to bananas fruits.

Imported bananas are harvested before the onset of enzymatic reactions (ripening), and their maturation is controlled artificial in special rooms using ethylene gas pumped from cylinders. This fastening of biological and biochemical processes during the artificial ripening of fruits can distort the characteristics of bananas with the loss of aroma and taste.

These results are based on numerous tests of non-organic bananas with emphasis to the analysis of ethylene from green stage to yellow bananas with brown flecks stage in two different conditions (biotic and abiotic).

The biotic conditions show the evolution of ethylene at four non-organic bananas stages and the abiotic conditions increases the production of ethylene at bananas.

The rapid development of LPAS method and its use for gas ethylene analysis shows that this technique is promising for studying the control mechanisms in fruit physiology such as those responsible for ripening, healing effects after wounding or post anaerobic injury.

As future work, more tests may provide more useful data to decipher the role of ethylene in the development of bananas fruit.

We have examined 4 mature organic and nonorganic champignon mushroom samples in synthetic air flow and nitrogen flow at atmospheric pressure.

Nonorganic champignon mushroom samples (33-35 g) were obtained from international dealers (supermarkets), produced in European countries.

The organic or nonorganic mushrooms used in these measurements were stored at 4^0C for subsequent use. Before starting the ethylene determination, all mushrooms were acclimatized over 1 h at room temperature (23 - 25^0 C) and then introduced into a small glass cuvette (with a volume of 150 cm^3) for measurements.

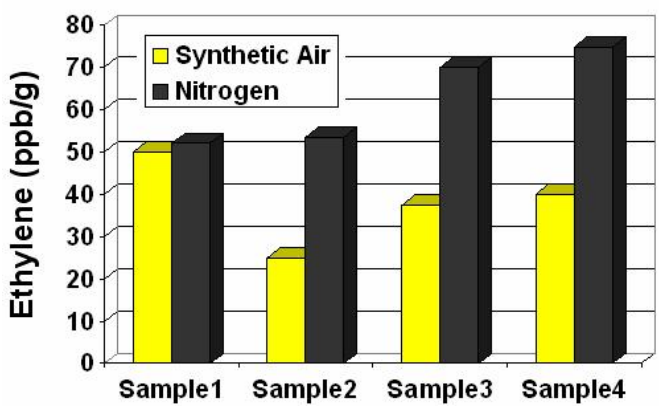


Fig. 14 - Ethylene amount in nonorganic champion mushrooms.

Organic champignon mushrooms cultivated organically were obtained from a local farmer (near Bucharest) and transported to the Laboratory for analysis. The organic cultivation area for harvested mushrooms was treated only with compost of animal manure (natural fertilizers) and the mushrooms were sorted, evaluated (at harvesting stage) and expressed in grams (between 33 g and 35 g).

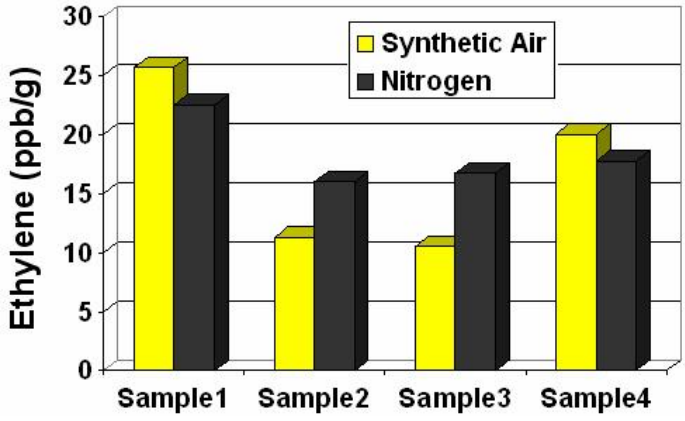


Fig. 15 - Ethylene amount in organic champion mushrooms.

The results from Figs 14 and 15 illustrate that when we applied nitrogen flow to organic and nonorganic champignon mushrooms (compared with synthetic air flow), the ethylene production is forced and starts to rise. Nitrogen can be one of the most critical nutrients in mushrooms production and can create quality problems promoting excessive development that depresses the organic and nonorganic Fungi.

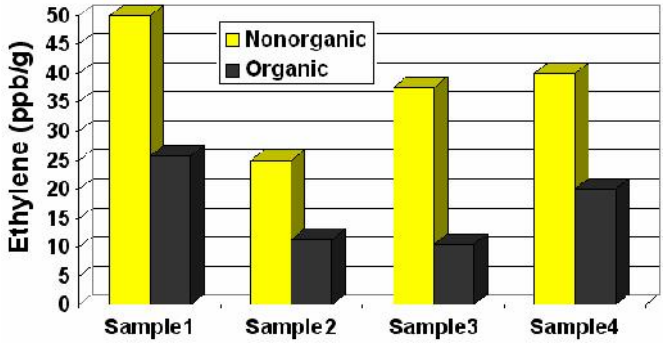


Fig. 16- Ethylene amount in synthetic air at organic and nonorganic champion mushrooms.

As a second observation of our measurements for all the mushrooms in synthetic air flow (shown in Fig. 16), we concluded that for all samples controlled with natural methods (organic), the ethylene level is lower compared to all champignon mushroom samples from supermarkets (nonorganic), where the concentration of ethylene rises by

more than 100%. \

Measurements were made to determine if the nonorganic champion mushrooms release more ethylene gas compared with organic ones. We assessed the effect of nitrogen flow in champion mushrooms quality using LPAS method.

Our measurements demonstrated that nonorganic mushrooms determine a greater increase of the ethylene concentration in the respiration of Fungi. As we can see in Figs. 14,15 and 16, there were quantitative changes in ethylene production between the mushrooms harvested from the two growing systems (organic and nonorganic). The level of the ethylene was about 100% higher for nonorganic mushrooms than for organic mushrooms farming. Such considerable differences could originate either from differences in nitrogen availability or from limitations to growth imposed by the more stressing conditions (like irradiation, toxicity or growth hormone) prevailing in nonorganic farming.

The results showed that the LPAS system could play an important role in testing the quality of mushrooms being able to distinguish between organic and nonorganic Fungi samples.

LPAS system has been demonstrated that it will play an important role in the future of exhaled breath analysis. The key attributes of this technique is sensitivity, selectivity, fast and real time response and ease to use. LPAS system is a sensitive, non-invasive and real time method to accurately analyze breathing ethylene and ammonia gas concentrations that possess high absorption strengths and a characteristic absorption pattern in the wavelength range of the CO_2 laser.

The applications of resonant PA spectroscopy include concentration measurements and trace gas analysis, accurate determinations of thermophysical properties, detections of dynamic processes such as gas mixing or chemical reactions, relaxation processes (determinations of collisional lifetimes of specified quantum states and routes of energy exchange in polyatomic molecules), spectroscopic experiments, studies of aerosols, etc. Trace-gas sensing is a rapidly developing field, in demand for applications such as process and air-quality measurements, atmospheric monitoring, breath diagnostics, biology and agriculture, chemistry, and security and workplace surveillance.

Project Manager,

Dr. Cristina ACHIM