

Article

# Monitoring of Post-Harvest Maturation Processes inside Stored Fruit Using Photoacoustic Gas Sensing Spectroscopy

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**Abstract:** Gases produced inside harvested fruit sensitively influence the continuing quality of the stored fruit and its maximum time of storability. In this work, the evolution of gaseous volatiles inside “Golden Delicious” apples were studied using CO<sub>2</sub> laser photoacoustic spectroscopy with the aim of developing new methods for in-storage fruit quality monitoring. Studying the concentrations of volatile organic compounds generated inside “Golden Delicious” apples during storage, it was found that the concentrations of these compounds depended on the stage of maturity reached during fruit maturation and on the conditions of preservation. Numerical simulations using COMSOL Multiphysics software were used to study the conversion of ethylene to ethanol in the course of respiration processes occurring inside stored food. Experimental data obtained by means of photoacoustic spectroscopy were used to critically assess the simulation results. Using the combination of both techniques, new prospects for the development and implementation of advanced schemes of fruit storage and preservation have emerged.

**Keywords:** gas detection; gas sensing techniques; volatile organic compounds; infrared photoacoustic spectroscopy; COMSOL Multiphysics

## 1. Introduction

Fruits are well recognized for their importance to human health as these are good sources of energy, vitamins and minerals without which the human body cannot maintain proper health and resilience to disease [1]. Proper fruit storage helps to reduce the chances of suffering food-borne illnesses. To ensure optimal storage conditions for long periods of time it is necessary to provide adequate external environments and to investigate those biochemical processes that go on within the stored fruit themselves [2]. External environmental factors that can affect fruit storage are light and temperature while the internal factors are gases and volatiles diffusing through fruit tissue and controlling its maturation. The internal atmosphere of fruit has importance to postharvest physiology and food science and technology. The involvement of gases like ethylene and ethanol in fruit development has a crucial effect on the fruit’s maturation and storability [3]. The correct timing of the harvest, the maturity and the aging of the apples, for instance, may be best determined by monitoring the biosynthesis of fruit ethylene.

During development and maturation, fruit exhibit two distinct respiratory patterns and which allow fruit to be classified into climacteric and non-climacteric groups [4–6].

Climacteric fruit are those that can ripen after being picked and which show a dramatic increase in the respiration rate during ripening. In contrast, non-climacteric fruit are characterized by the impossibility of ripening once they have been removed from the plant [7,8].

Apple is a climacteric fruit and most of the relevant physiological changes are triggered and governed by the action of ethylene. To prolong the storage period, the maturation must be delayed by sorting, but also by storing the fruit at low temperatures and in controlled atmospheres. Under proper conditions and harvested at optimum storage potential, apples may be stored as long as 12 months [9].

The volatiles present inside the fruit are ethylene, ethanol, acetaldehyde, methanol, acetone, butanol, propanol, ethane, hexanol, sulfur compounds, ammonia, etc. These volatiles can be used to determine the optimum maturity stage for the harvesting of climacteric fruit. It is important to evaluate the significance of volatiles and their interaction with ethylene in postharvest situations. Different factors and conditions can influence the accumulation and release of these volatiles [10]. The volatile plant hormone ethylene is a hydrocarbon gas without scent and invisible to the eye. In fruit, ethylene is a gas emitted naturally in the process of ripening or produced upon inflicting plant injury. This potent molecule regulates the plant's growth and development as well as the speed of this processes [11–15] at concentrations as low as about 0.01 to 1.0 parts per million (ppm). Climacteric products, such as tomatoes and apples, can produce tens of ppm of ethylene. Recent research aiming at improving the postharvest fruit quality, therefore, concentrates on the possibility of controlling the effect of ethylene [16,17].

Ethanol is a simple alcohol resulting from the fermentation of fruit sugars by yeast and occurs when O<sub>2</sub> concentrations are very low [18]. The maturation of the fruit causes changes in the levels of O<sub>2</sub> and CO<sub>2</sub> within the fruit and these changes are usually accompanied by a net reduction of the O<sub>2</sub> to CO<sub>2</sub> ratio and by the accumulation of ethanol inside the fruit. The beneficial effects of low atmospheres of high O<sub>2</sub> and CO<sub>2</sub> inside the fruit lead to a reduction in the respiration rate, ethylene production and harmful effects, such as ethanol accumulation. Detecting ethanol in an internal fruit atmosphere may provide an additional measure of maturity.

While ethylene and ethanol regulates the fruit's growth, ammonia is a common byproduct of the metabolism of the nitrogenous compounds and is involved (with proteins) in plant urea metabolism. The urea pathway has been studied not only in mammals and amphibians, but also in birds, invertebrates, insects, plants, yeast, fungi and even microorganisms. Compared to urea, ammonia is smaller, more volatile and more mobile [19].

Over a number of years, diverse techniques were utilized to identify volatile organic compounds created by living organisms under a variety of conditions. These methods for gas detection include different analytical tools [20], but techniques based on laser systems are used successfully in many directions of research [15,19,20].

Laser photoacoustic spectroscopy (LPAS) has the ability to monitor trace gases with high importance for biologic and medical applications. Because it is a sensitive and selective technique, it has limits ranging from the part-per-million (ppm,  $\mu\text{mol/mol}$ ) to less than part-per-billion (ppb,  $\text{nmol/mol}$ ) and is able to detect one or several gases and is used successfully increasing in fields [20,21]. The objective of this study is demonstrating the potential of laser-based photoacoustic spectroscopy in combination with multiphysics numerical simulation in detecting volatile organic compounds, such as ethylene, ammonia and ethanol emitted from Golden Apples during their maturation process.

## 2. Materials and Methods

In the present study, we performed analyses on "Golden Delicious". The apples were taken from the supermarket with the specifications: Country of origin: Romania, Origin: Voinești-Dâmbovița.

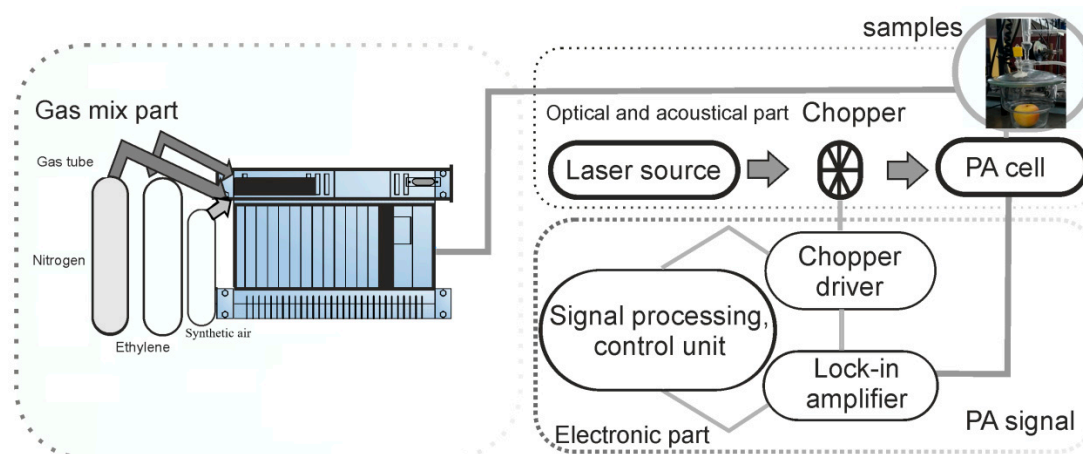
The fruit are yellow in color, with rust spots; the pulp is yellowish, crispy with a pleasant aroma, medium strength and medium fruit size 133 g, spherical-conical shape. The "Golden Delicious" apples are preferred by most consumers.

Fruits were obtained from international producers (supermarkets). All apple fruit used in these measurements were stored at room temperature (25–27 °C) for subsequent use. The fruit were imported into a glass cuvette with a volume of 150 cm<sup>3</sup> at room temperature and then connected to the LPAS

system (a homemade system). This system is used for the sensitive detection of ethylene, ethanol and ammonia from the fruit to explore the role of these volatiles in fruit development.

CO<sub>2</sub> laser photoacoustic spectroscopy has known a great increase over the years, in principal by enlarging the application fields gaining importance when it is applied to life sciences being able to detect a minimum concentration of 0.9 ppbV.

The continuous wave CO<sub>2</sub> laser (LIR 25 SF, a homemade laser) line-tunable, frequency stabilized emitting radiation in the 9.2–10.8 μm region on 54 different vibrational–rotational lines with powers varying between 0.5 and 6.5 W [19,22]. The CO<sub>2</sub> laser beam (a homemade laser) is modulated in intensity by a mechanical chopper operating at the appropriate resonant frequency of the cell (564 Hz), is focused by a ZnSe lens (homemade lens) and then introduced in the photoacoustic cell provided with four microphones where the acoustic wave is detected and to generate a corresponding electrical output signal. This signal is fed into a lock-in amplifier (Stanford Research Systems model SRB30 DSP, Sunnyvale, CA, USA) that gives the amplitude and the phase of the photoacoustic signal. A power meter measures the laser beam power after the photoacoustic cell. Its digital output is plugged in the data acquisition interface module along with the output from a lock-in amplifier. All experimental data are processed and stored by a computer (Figure 1).



**Figure 1.** Block diagram of the CO<sub>2</sub> laser photoacoustic system.

Gas handling and vacuum systems are used to provide gas purity in the photoacoustic cell, to pump out the cell, set the sample gas in the photoacoustic cell at a controlled flow rate and monitor the pressures of gas mixtures. The gas handling system also includes two gas flow controllers (MKS Instruments Deutschland GmbH, MÜNCHEN, Germany) which limit the transfer of the sample in the cell to a flow rate of 36 L/h (600 sccm: standard cubic centimeters per minute) and the cuvette where the fruit is placed in order to be analyzed.

The CO<sub>2</sub> photoacoustic spectroscopy system is characterized by the resonance frequency  $f = 564$  Hz, quality factor of the system  $Q = 16.1$ , cell constant  $C = 5.41 \times 10^3$  (Pa·cm/W) cell, microphones responsivity  $4 \times 20 \times 10^{-3} = 8 \times 10^{-2}$  (V/Pa), responsivity  $R = 433$  cm·V/W. The pressure of the gas inside the cell influences the responsivity  $R$  of the photoacoustic cell. The laser beam interacts with the gas sent to the PA cell. The minimum detectable concentration of ethylene, ethanol or ammonia can be deduced as the ratio between minimum measurable voltage signal and the product of absorption coefficient, laser power and responsivity  $c_{\min} = V_{\min}/\alpha R P_L$ , where  $V$  (V) is the photoacoustic signal,  $\alpha$  (cm<sup>-1</sup> atm<sup>-1</sup>) is the absorption coefficient at the laser wavelength,  $c$  (atm) represents the trace gas concentration (usually given in units of per cent, ppmV, ppbV or pptV),  $P_L$  (W) is the unchopped laser beam power and  $R$  (V·cm/W) is the responsivity of the PA-photoacoustic cell [19–23].

The CO<sub>2</sub> laser photoacoustic spectroscopy system [19,20,22] was used to quantify the production of ethylene, ethanol and ammonia that are normally produced by apple fruit in normal aerobic conditions (synthetic air flow) over time (day 1, 7, 14, 21, 28 and 35, see Figure 2).



**Figure 2.** Pictures representing the evolution of a Golden Delicious apple over 35 days. The external appearance of apples that continues to degrade presenting speckles and wrinkles.

To evaluate the fruit tissue signal response from the glass cuvette (connected to the photoacoustic cavity (homemade cavity)), we carefully removed all kinds of residual volatiles from the gas line connecting the gas flow system with the PA cell by flushing this line with pure nitrogen at atmospheric pressure for a few minutes. After the system was flushed, we transferred the gas from the glass cuvette to the PA cell with a flow of pure synthetic air.

Performing trace gas detection by photoacoustic spectroscopy requires precise knowledge of the absorption spectrum of each gas species to be determined. For a given concentration of that gas, the absorption spectrum is obtained by measuring the photoacoustic signal over all available laser source wavelengths. Measurements of absorption coefficients for all wavelengths of the laser source facilitate the determination of maximum absorption coefficient and permits to detect with high accuracy individual trace gas concentrations from a multicomponent mixture.

The assessment of absorption coefficients of ethylene, ethanol and ammonia was performed for specific CO<sub>2</sub> laser lines. The PA cell was filled at atmospheric pressure with certified gas mixtures of 0.996 ppmV ethylene in nitrogen and, 10.5 ppmV ethanol in nitrogen and, 10 ppm ammonia in nitrogen. Using the software user interface that allows recording the laser power, the PA signal and the calculated absorption coefficients on different panels we can determine the specific line where ethylene, ammonia and ethanol have their maximum absorption coefficients.

Transpiration is the process by which fresh fruit lose moisture, processes that include the transport of moisture through the fruit skin and evaporation to the surrounding air. Metabolic activity in fresh fruit continues for a short period after harvest. In the present work we simulate the chemical reaction between ethylene and water vapors using Chemical Reaction Engineering Module from Comsol 5.1 [24]. The main goal of this analysis is to validate the LPAS measurements accuracy by evaluation the reaction results. In the gas phase there is a reversible chemical reaction between ethylene, water vapor and ethanol (see Equation (1))



The chemical reaction was implemented using input parameters those used in the LPAS measurements, such as pressure, temperature, the cuvette volume. We solve the model using a time-dependent study step.

### 3. Results

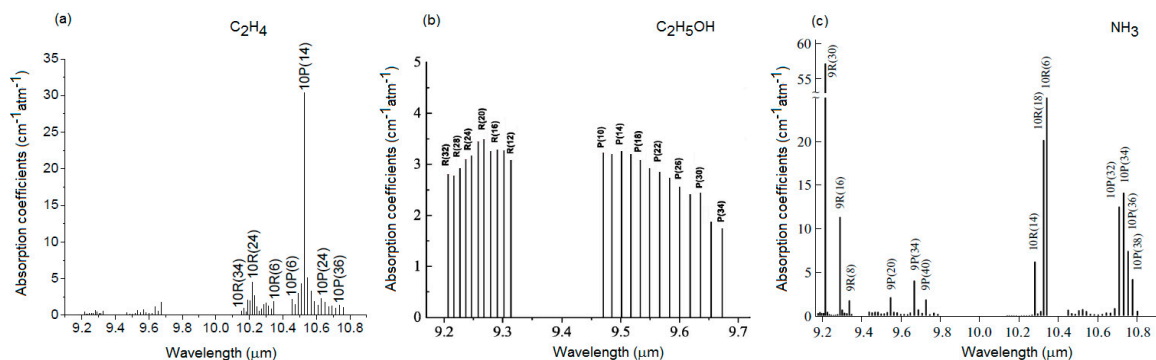
#### 3.1. Gas Detection Using CO<sub>2</sub> Photoacoustic Spectroscopy

The physiological response of fruit under aerobic conditions was investigated using CO<sub>2</sub> photoacoustic spectroscopy in order to evaluate the ethylene, ethanol and ammonia gas concentrations that were produced by golden apple fruit in the different stages of ripening.

The volatiles were investigated for six “Golden Delicious” apples over 35 days. Long-term experiments provide insight into the processes that produce changes in mature fruit and helped us to understand the significance of volatiles that differ in the rate of production under non-stressful conditions.

Ethylene, ethanol and ammonia emissions were established by introducing “Golden Delicious” apples into the cuvette sample and flushed with synthetic airflow at atmospheric pressure, and the

resulting gas from the glass samples was transferred in the cell and analyzed. For each gas detection, the laser lines were chosen at the wavelengths where the gas to be analyzed has the maximum absorption coefficient (Figure 3).

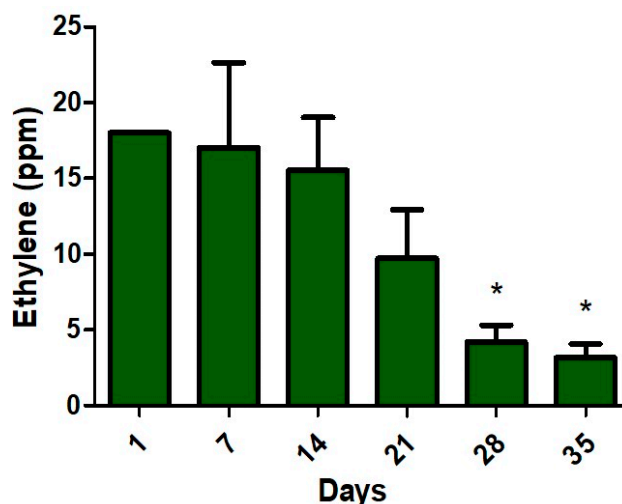


**Figure 3.** Absorption spectra of gases at CO<sub>2</sub> laser wavelengths. (a) Absorption spectra of ethylene, (b) Absorption spectra of ethanol, (c) Absorption spectra of ammonia.

The carbon dioxide laser operates in rotational-vibrational transition. Each vibrational transition consist of bands of discrete wavelengths resulting from a manifold of rotational structures, where lasing action can be obtained on more than a hundred lines. This laser operates in the middle infrared wavelength region with the principal wavelength bands centering around 9.4 and 10.6  $\mu\text{m}$ . The CO<sub>2</sub> laser transitions are 961  $\text{cm}^{-1}$  transition of the 1.4  $\mu\text{m}$  band and 1064  $\text{cm}^{-1}$  transition of the 9.4  $\mu\text{m}$  band. Because of the symmetry of the CO<sub>2</sub> molecule, laser transitions occur to lower energy levels whose rotational numbers are even, resulting in more than 30 lines in each of the two branches P and R.

For ethylene detection laser line was determined at 949.479  $\text{cm}^{-1}$ , where we have the maximum absorption coefficient of 30.4  $\text{cm}^{-1} \text{atm}^{-1}$  [19,20]. Ethanol was determined at 9R(22) laser line with a maximum absorption coefficient of  $\alpha = 4.081 \text{ cm}^{-1} \text{atm}^{-1}$  [20,23] and the maximum absorption coefficient of ammonia  $\alpha = 57.1 \text{ cm}^{-1} \text{atm}^{-1}$  was established at 9R(30) [22].

Figure 4 shows the level of ethylene gases emitted by all six “Golden Delicious” apple fruit ranging from an initial weight of about 133 g to 89 g at the end of the experiment.



**Figure 4.** Ethylene concentrations emitted from “Golden Delicious” apples over 35 days under normal conditions. The error bar for day 1 is not visible due to a very small error. \*  $p < 0.05$  for unpaired  $t$ -test with Welch’s correction.

All the data are presented as mean  $\pm$  standard deviation if not stated otherwise, from at least 4 independent experiments. The statistical analysis was performed using GraphPad Prism 5 software

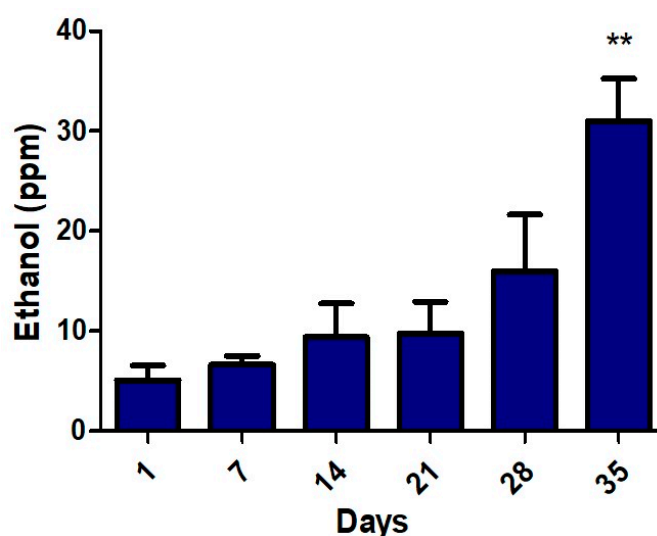
(GraphPad Software, Inc., San Diego, CA, USA) and applying the unpaired *t*-test with Welch's correction.  $p < 0.05$  was considered statistically significant.

Starting with day 28 the ethylene concentration decreases, the apples become speckled and wrinkled.

During the experiment, a degradation of the apples can be observed, evidenced by the decrease of the concentration of ethylene, the staining and the wrinkling of the apple, but also by the weight loss of the apples.

The ethanol emission was measured at the same time with ethylene by changing the laser line. Ethanol molecules present a strong absorption 9R(22) laser line where the absorption coefficient is  $\alpha = 4.081 \text{ cm}^{-1} \text{ atm}^{-1}$ .

In Figure 5 the release of ethanol molecules over 35 days was recorded and the process of degradation was studied.



**Figure 5.** Ethanol concentrations emitted from “Golden Delicious” apples over 35 days under normal conditions. \*\*  $p < 0.01$  for unpaired *t*-test with Welch's correction.

In all cases the ethanol presents a constant increase in concentration providing an early indicator for fermentation. At the end of the experiment when the apple present spots and wrinkles a maximum in ethanol emission was observed.

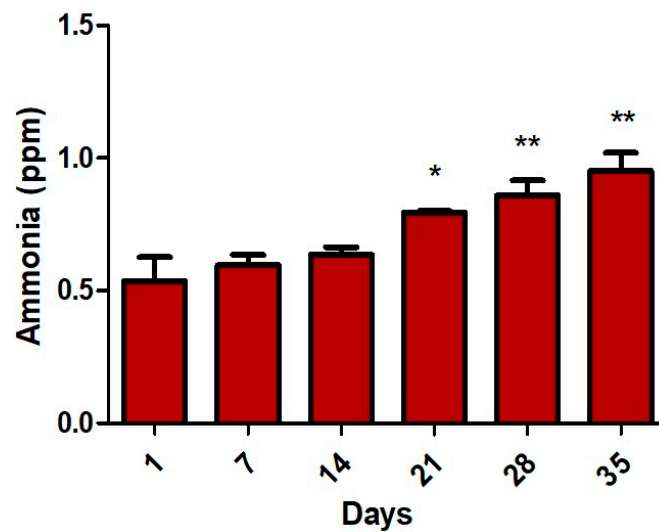
The experiment reveals that the level of ethanol increases in day 35 when the ethylene concentration is minimal. The production of ethanol stops the process of fruit ripening.

During fruit maturation, the ethanol accumulates at low levels and plays an important role in the postharvest stage of apples. From our experiments, it can be observed that the ethanol emission is steadily increasing with apple degradation reaching a maximum value of 34 ppm on day 35.

The ammonia concentration from apples was measured at the same time with ethylene and ethanol by changing the laser line to 9R(30) laser line where the ammonia absorption coefficient is  $\alpha = 57.1 \text{ cm}^{-1} \text{ atm}^{-1}$ .

Figure 6 shows the level of the ammonia gases emitted by apple fruit over 35 days under normal conditions.

It can be observed that the ammonia content in the internal atmosphere over 35 days slowly increases starting with day 21.



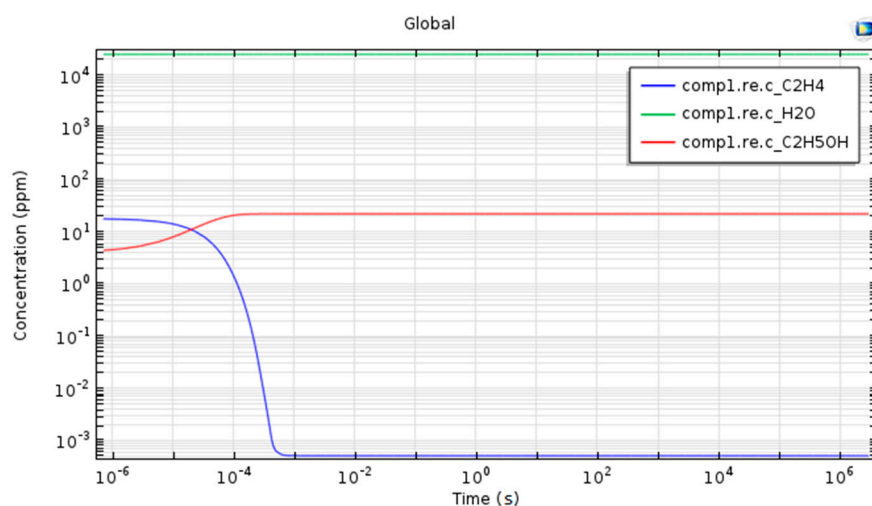
**Figure 6.** Ammonia concentrations emitted from “Golden Delicious” apples over 35 days under normal conditions. \*  $p < 0.05$ , \*\*  $p < 0.01$  for unpaired  $t$ -test with Welch’s correction.

### 3.2. Numerical Simulation and Experimental Verification of Chemical Reactions

Using numerical simulations, we studied whether there is a possible chemical reaction between ethylene and water vapors found in apple respiration, a reaction that could influence the concentrations of ethylene and ethanol.

The numerical study was performed depending on time, the chosen interval being 1:35 days, interval identical to the one in which the LPAS measurements were realized. In addition, the numerical simulations were performed in the same temperature and pressure conditions, at room temperature and atmospheric pressure.

Figure 7 shows the time evolution of ethylene, ethanol and water vapors concentrations over a period of 35 days.



**Figure 7.** Time dependence of ethylene, ethanol and water vapor concentrations plotted on a logarithmic scale over a period of 35 days.

The results obtained from the numerical simulations show that there is no chemical reaction that influences the concentrations of ethylene and ethanol in apple respiration at room temperature and atmospheric pressure and the concentrations of these two gases are due to the respiration process in

apples. In the conditions that were carried out in the experiments, all gases from the apple respiration behave independently.

#### 4. Discussion

The current research describes an experimental study of the detection of ethylene, ethanol and ammonia released by Golden Delicious apples using laser photoacoustic spectroscopy. This technique is very precise allowing each gas to be measured with high efficiency. In a complex mixture of gases, a proper absorption laser line must be chosen for the determination of the individual concentration so that there are little or no interferences with other gases [24–28].

The detection of ethylene, ethanol and ammonia from apples presents interest for better understand internal fruit development in storage conditions. These volatiles have important roles in the maturation of apples. Ethylene is an important factor that produces changes in climacteric fruit, but ethanol and ammonia also have their influences.

Ethanol is capable of retarding senescence and inhibiting ethylene production in plants, leading to injury symptoms in fruit. Ammonia is a product of protein catabolism associated with the senescence of fruit and it accumulates during postharvest handling.

During 35-day trials, golden apple ethylene emissions showed a typical climacteric growth during the first seven days and then began to fall, while the ethanol production of apples increased gradually.

From day 21 on, apples creased and lost weight. Starting on day 28, the apples becomes speckled and wrinkled, at day 35 their weight had dropped to about 89 g.

From our measurements on ethylene phytohormone analysis, we can observe that ethylene emission is lower when the apples are more speckled and wrinkled. The ethanol normally accumulates at low levels during fruit maturation and plays an important role in the postharvest stage of apples. An important aspect of the study on the internal processes of fruit is the emission of ethanol that increases constantly with the degradation of the apple reaching a maximum value of 34 ppm on day 35. Ammonia pile-up inside the stored fruit slowly increases over the 35 days period causing fruit spoilage and skin blackening.

The principal finding from our investigation is that at day 28, when the ethylene emission drops significantly and when ethanol emission increases sharply, is the point when apple development becomes suppressed. The ethylene decreases from 18 ppm to 4.2 ppm (77%) and the ethanol increases from 5 ppm to 16 ppm (220%).

A considerable number of investigations were conducted on the specific properties of the aging and ripening of fruit [28–35]. Ethylene, ethanol and ammonia emission from apples influences the internal activity of fruit leading also to a modification of the outer layer.

At the same time, we determined through numerical simulations using Comsol Multiphysics the conversion of ethylene to ethanol during the respiration of apples for a period of time. The simulation results were compared with those obtained by photoacoustic spectroscopy.

From a practical point of view, information about ethylene biosynthesis, ethanol and ammonia action in the internal apple atmosphere reported in this study can be used to create effective tools capable of evaluating and predicting the evolution of the degradation process. New opportunities can be developed for researchers to promote new and improved ways for nondestructive quality evaluation of fruit using gas sensing techniques.

#### 5. Conclusions

In summary, CO<sub>2</sub> laser photoacoustic spectroscopy was performed to give information about the internal processes occurring in Golden Delicious apples stored over long periods of time. At the same time, the conversion of ethylene to ethanol during respiration of apples was determined through numerical simulations using Multiphysics and compared with data obtained by photoacoustic spectroscopy.



Detection of these gases (ethylene, ethanol and ammonia) from apples using CO<sub>2</sub> laser photoacoustic spectroscopy hold promise for better understanding internal fruit development in storage conditions. These volatiles have important and different roles in apple processes. The main benefit of CO<sub>2</sub> laser photoacoustic spectroscopy on the “Golden Delicious” apple’s internal atmosphere is that has the ability to determine the low concentration of ethylene, ethanol and ammonia. It was found that as the concentration of ethanol increased, ethylene production decreased after reaching a maximum of 23 ppm—and ammonia slowly increased. Assessment in the endogenous volatile compounds from the fruit may demonstrate that the quality and quantity of volatiles may be linked to postharvest management of fruit. New information about these volatiles will help us in achieving the best shelf life of fruit.

Very little is known about the influence of different volatiles in regulating the processes in fruit. Ethylene remains a major controlling factor in climacteric fruit processes, but its biosynthesis, perception, sensitivity are influenced by other endogenous volatiles. Finding as much information about these volatiles as possible will help us in achieving the best shelf life of fruit.

Developing fruit-specific and real-time systems to manipulate levels of important gases during storage will allow further improvements in shelf life and quality of stored fruit to be attained.

Overall, the results of this research show that it is possible to assess volatile molecules that can further our understanding of postharvest management processes of fruit.

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**Conflicts of Interest:** The authors declare no conflicts of interest.

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