Detection of ethanol and acetaldehyde released from cabbage seeds of different quality: Laser photoacoustic spectroscopy versus FTIR and headspace gas chromatography

D. Bicanic[a)]
Laser Photoacoustic Laboratory, Division of Biophysics, Department of Agrotechnology and Food Sciences, Wageningen University and Research Centre, Dreijenlaan 3, 6703 HA, Wageningen, The Netherlands

S. Persijn[b)]
Life Science Trace Gas Exchange Facility, Department of Molecular and Laser Physics, University of Nijmegen, Toernooiveld 6525 ED Nijmegen, The Netherlands

A. Taylor
Department of Horticultural Sciences NYSAES, Cornell University, Geneva, New York 14456

J. Cozijnsen
Wageningen University and Research Centre, Department of Food Chemistry, Department of Agrotechnology and Food Sciences, Wageningen University and Research Centre Bomenweg 3, 6703 HD Wageningen, The Netherlands

B. van Veldhuyzen
Organic Chemistry Division, Department of Agrotechnology and Food Sciences, Wageningen University and Research Centre, Dreijenplein 2, 6703 HD Wageningen, The Netherlands

G. Lenssen
Rijk Zwaan Zaadteelt en Zaadhandel B.V., de Lier, The Netherlands

H. Wegh
Environmental Technology Division, Department of Agrotechnology and Food Sciences, Wageningen University and Research Centre Bomenweg 3, 6703 HD Wageningen, The Netherlands

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Detecting early ageing stages of seeds in storage is of great concern to vegetable seed companies. Despite their reliability conventional germination tests are destructive and time consuming. One alternative towards assessing the quality of seed is to monitor the concentration of certain volatiles (acting as biological markers) evolved from the seeds; examples are ethanol (EtOH), acetaldehyde, ethane, methanol, etc. Most of the presently used methods have only moderate insensitivity and are therefore not suitable for the on-line measurements. In this work CO laser photoacoustic spectroscopy (LPAS) was used to investigate the on-line evolution of EtOH and acetaldehyde from the imbibed nonaged and aged seeds of cabbage. The overall performance of LPAS was superior to that of Fourier transform infrared and of gas chromatography. © *2003 American Institute of Physics.* [DOI: 10.1063/1.1512775]

I. INTRODUCTION

Vegetable seed companies seek the availability of rapid methods capable of detecting the early ageing stages of seeds in storage. The standard germination test, a truly reliable, but destructive technique, requires a long time interval before the outcome of the test is known. An alternative route towards the quality assessment of seeds is to study volatiles evolving from seeds. For example, ethanol (EtOH) and acetaldehyde biochemically synthesized with respiration are released from dry and hydrated seeds. Early research has shown that the evolution of EtOH and acetaldehyde from the low quality seeds exceeds that characteristic for the high quality seeds.[1] Therefore volatile compounds that are rapidly synthesized in early germination could serve as candidate markers of seed quality.

The majority of studies with the objective to detect volatiles from crop seeds was conducted by means of gas chromatography (GC) using a traditional flame ionization detector or the immobilized enzyme technology.[1,2] A drawback of these approaches is their inability for the on-line detection of compounds. In recent years, laser based photoacoustic spectroscopy (LPAS) has emerged as a new and powerful tool for a real-time, highly sensitive, and specific concentration monitoring of various gases.[3–5] The availability of intense infrared lasers makes the LPAS a candidate method for observation of low synthesis rates of volatiles released from aged and nonaged seeds. This has prompted us to propose the LPAS study of EtOH and acetaldehyde emitted from the cabbage seeds previously exposed to a controlled ageing

a)Electronic mail: dane.bicanic@user.aenf.wau.nl
b)Present address: Soegijapranata Catholic University, Faculty of Agricultural Technology, Semarang, Indonesia.

treatment. The results obtained by LPAS were compared to those acquired by Fourier transform infrared (FTIR) with a HgCdTe detector and the headspace analysis using GC equipped with the flame ionization detector.

II. EXPERIMENT

Seeds originating from one seed lot of ''Green Coronet'' cabbage (*Brassica oleracea* L. Group. Capitata) were kindly provided by American Takii Inc., Salinas, CA. Different seed quality levels were prepared using seeds from the same seed lot by aging them in the laboratory in two independent steps. Seeds were first equilibrated to 70% relative humidity, heat-sealed in aluminum-foil plastic-laminate packets and then aged at 40 °C for 0, 14, and 28 days.[2] Following each ageing period, the quality of seed was assessed as based on the outcome of the standard germination test conducted at 25 °C. Seeds were kept in a refrigerator (about 5 °C) in sealed Ziplock baggies. Prior to opening the baggies were allowed to warm up for about an hour to avoid possible condensation.

To obtain large concentrations of volatiles within a relatively short time, aged seeds (initial moisture content 6.5% by mass) were partially imbibed. The amount of distilled water added to seeds (72 μl to 0.2 g corresponding to a 1:2.7 water-to-seed ratio by mass) hydrated them to 0.45 MC_f (MC_f is the percent moisture content when seeds are fully hydrated). At 0.45 MC_f seeds are both hydrated and in an aerobic condition.

Low quality seeds produce more EtOH and acetaldehyde than high quality seeds; this is due to fermentation associated with seed ageing.[3] Acetaldehyde, an intermediate compound in the formation of EtOH is expected to rise initially and then decrease with time after hydration. Most measurements are made after 6 and more commonly 24 h after hydration.[3] However, this article reports LPAS measurements of EtOH and acetaldehyde after only 3 h of imbibition.

A. LPAS

The PAS study was performed using three resonant (1.3 kHz) PA cells (the volume of each was 26 ml) placed behind one another and inside the cavity of a cryogenic CO laser the intensity of which was modulated by a mechanical chopper. Imbibed seeds of different ages were distributed in three separate glass ''reservoirs;'' each of them was connected with one PA cell. The transport of volatiles accumulated in the headspace above the seeds towards the PA cell was achieved by a flow (2 l/h) of conditioned air.

During the initial 3 h of imbibition (the uptake of liquid water by seeds was completed) the PA cells were flushed continuously with dry air. Then reservoirs were connected to PA cells and the carrier gas diverted to flow above the seeds. The PA signals were measured at six preselected CO laser lines: three of them coincided with the absorption lines of acetaldehyde, EtOH, and water, respectively, while the remaining ones were used to estimate the level of a background signal. The measuring sequence implied tuning of the laser, optimization of power, and recording of PA signals from either of the three cells; this sequence was repeated three times. What follows is the measurement of laser power, averaging, and normalization of PA signals. After 40 s the laser is tuned to another emission line and the whole procedure repeated; present resolution is 4 min. The interference of longer alcohols and of other large molecules was

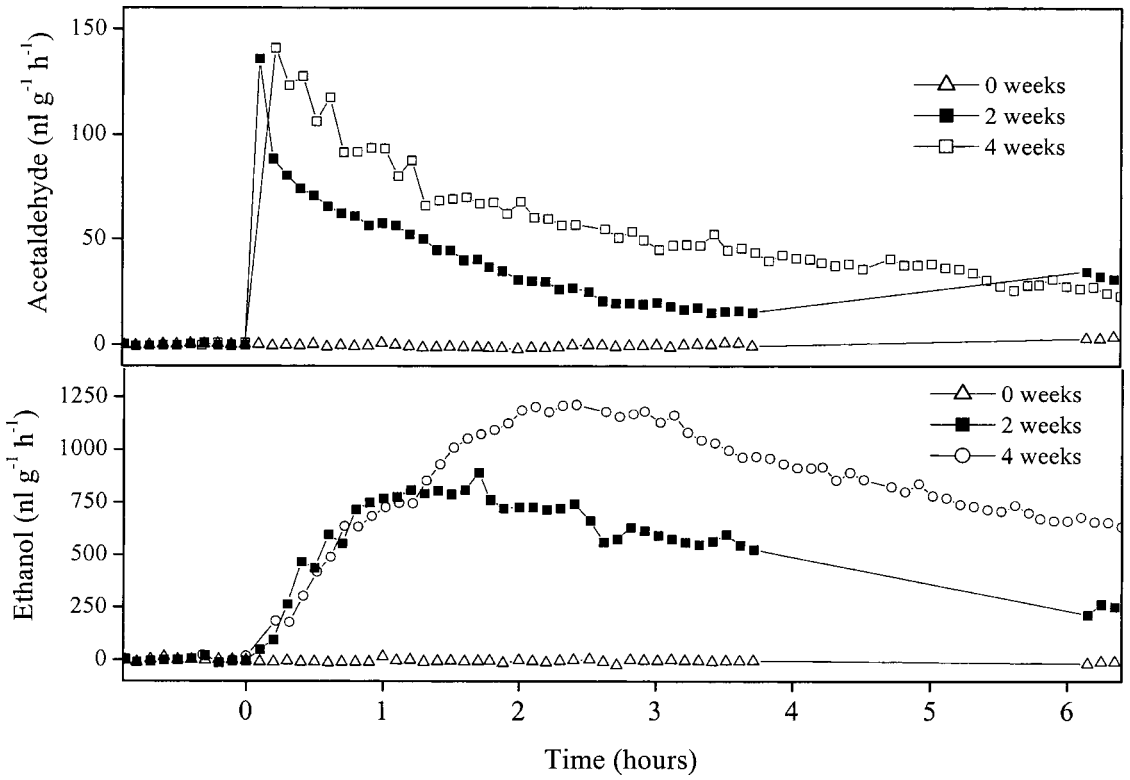

FIG. 1. The production rates of acetaldehyde (top) and EtOH (bottom) from imbibed cabbage seeds of different quality. The $t=0$ on the x axis corresponds to 3 h of imbibition.

avoided by using the cold trap. The response of the LPAS system was calibrated using a 10 ppmv mixture of ethanol in nitrogen.

B. FTIR

Unlike LPAS that operates in the flow through regime, GC and FTIR use a closed system that requires ample quantities of air because seeds need oxygen to respire. If for a given weight of seeds the headspace above the seeds is too small, oxygen will be depleted (anaerobic fermentation) leading to substantial production of EtOH even from high quality seeds. Accommodating 0.2 g seeds within 10 ml air space is usually recommended.

Three homemade cylindrical FTIR cells (125 ml each) were constructed from Pyrex glass and sealed with a pair of KCl windows. To reach the targeted 0.45 MC_f each cell was loaded with 2 g seeds (aged for either 0, 2, or 4 weeks) and 720 μl distilled water added. Under these conditions the above-mentioned criterion (0.2 g seeds within 10 ml air space) for avoiding hypoxia is met. The concentration of EtOH was monitored at 1394.22 cm^{-1}; at this wave number the absorption is quite strong (absorption coefficient of EtOH vapor is 17 $atm^{-1}\,cm^{-1}$).

C. Gas chromatography

Seeds were hydrated to 0.45 MC_f (0.2 g seeds added to 72 μl distilled water) and kept in a 10 ml vial. Following imbibition seeds were incubated and agitated for 10 min in the glass container maintained at 60 °C. Two millilitres of the headspace (above the seeds) were then withdrawn and injected on DB Carbowax GC column.

III. RESULTS AND DISCUSSION

A. LPAS

Figure 1 shows the production rates (in nl/g/h) of EtOH and acetaldehyde recorded on-line (gas carrier flow 2 l/h) from 1 g nonaged and aged cabbage seeds. The zero of the time axis coincides with a 3 h long imbibition period; at $t=0$ the reservoirs with seeds were actually connected to PA cells. The acetaldehyde peak appears before that of EtOH; furthermore, the concentration of EtOH is significantly higher than that of acetaldehyde.

The integrated production of EtOH and acetaldehyde for the same seeds is shown in Fig. 2. While EtOH production from nonaged seeds remains practically zero, that of seeds aged for two weeks is 3.6 and 5.9 μg/g after 3 and 6 h, respectively. For cabbage seeds aged for four weeks, production is higher i.e., 4.9 and 9.7 μg/g after 3 and 6 h, respectfully. As to the longer periods, the production rate of EOH begins to level off reaching 11.4 μg/g (after 9 h), 14.4 μg/g (after 12 h), and 15.4 μg/g (after 15 h).

B. FTIR

Before commencing the actual FTIR experiment on the cabbage seeds one has attempted to estimate the detection limit (EtOH) of a typical FTIR instrument. This was accomplished by injecting 2 μl liquid EtOH directly into a 125 ml

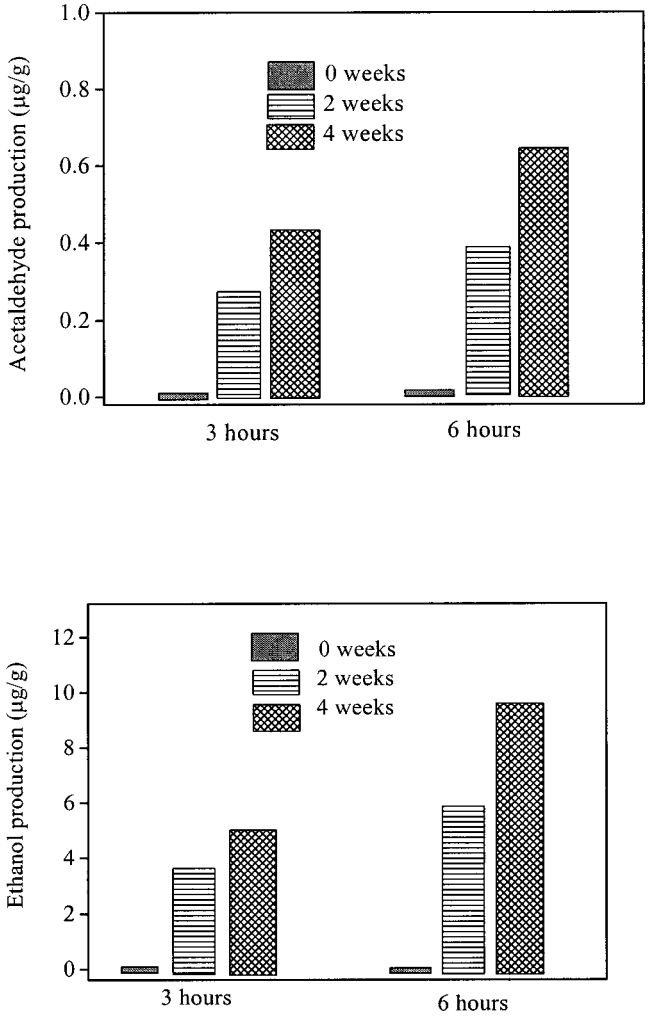

FIG. 2. The integrated production (expressed in μg gas per gram seed) of EtOH (bottom) and acetaldehyde for imbibed cabbage seeds of different quality.

FTIR cell. Assuming complete evaporation, 2 μl of EtOH liquid will convert to 860 μl of EtOH gas. In the 125 ml FTIR cell this is equivalent to 6700 ppmv (at room temperature); under static conditions the signal-to-noise ratio was approximately 6 at 1394.22 cm^{-1}.

No evidence for EtOH presence in the FTIR cell was found during a 2 week long period following the onset of the experiment. Bearing in mind (i) rather low (typically 1 μl/g h) production rate of EtOH (as observed in LPAS experiment) from imbibed and aged cabbage seeds and (ii) the sensitivity based on the outcome of the study mentioned above, it is likely that long periods are needed before the concentration of EtOH accumulated in the cell reaches the level at which it becomes measurable for a HgCdTe detector.

C. Gas chromatography

No EtOH from the nonaged, wetted (0.45 MC_f) cabbage seeds was found even after 2 days of imbibition. For the aged seeds EtOH production is apparently very low reaching 1.85 μg/g (seeds aged for 2 weeks) and 1.65 μg/g (seeds aged 4 weeks) after 6 h. These values are close to 3.8 and 4.9 μg/g found for the same seeds in the LPAS experiment. Sur-

prisingly, the production of EtOH, as measured by the GC shows a decreasing trend during the next 36 h. No comparison with the outcome of the LPAS study could be made as the latter was terminated after 9 h. The outcome of an independent GC study carried out on the same seeds but imbibed to a higher level (water-to-seed ratio higher than 1:2.7) or submerged (water-to-seed ratio exceeding 8:1), indicates a clear correlation between the production of EtOH and duration of imbibition period.

IV. CONCLUSION

Ethanol is the major volatile produced by imbibed (water-to-seed ratio 1:2.7) aged cabbage seeds. Acetaldehyde is an intermediate; after the initial increase its concentration declines as it is enzymatically oxidized to EtOH. Despite operating in a dynamic mode, the LPAS was demonstrated to be more sensitive than FTIR in detecting EtOH as a biochemical marker. The results obtained by LPAS and those acquired by GC agree fairly well but only during the initial few hours of imbibition. Furthermore, GC data suggest that the production of EtOH from seeds decreases at prolonged times (2 days). It is possible that an accumulation of EtOH could impair the physiology of the seeds as EtOH has a fluidizing effect on cell membranes at high concentrations. Since the present LPAS study was restricted to 9 h following the imbibition, no direct comparison with the GC data could yet be made.

The analytical methods differ actually in the sampling techniques. Unlike FTIR and GC headspace analysis that both operate in a static regime, or the enzymatic detector that measures true EtOH concentration within the seeds, the LPAS measures the concentration difference $c_{\text{in}} - c_{\text{out}}$ (where c_{in} and c_{out} represent the concentrations of EtOH inside and outside the seeds), i.e., EtOH is generated and removed concurrently. It is possible that establishing the gradient and removing the volatiles may change the absolute amount of EtOH produced.

In conclusion, LPAS is a highly sensitive technique for on-line monitoring of EtOH and acetaldehyde released from aged, imbibed seeds. Present sensitivity of the LPAS method allows measurements on seeds to be performed 3 h after the onset of imbibition and even sooner. The enhancement of sensitivity is anticipated if the LPAS experiment is conducted at 1066.56 cm^{-1} using the CO_2 laser for the excitation. Although at 1066.56 cm^{-1} the absorption of EtOH vapor is weaker (absorption coefficient 2.8 $atm^{-1}\,cm^{-1}$) than that at 1392.44 cm^{-1}, the output power of the CO_2 laser line at this wave number is substantially higher than that of the CO laser. Furthermore, the development of new powerful coherent sources of infrared radiation will and the optimization of the PA cell design might eventually enable one to study low production rates of volatile markers even from dry seeds; work on this matter is already in progress.

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