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Photoacoustic Continuous Detection of Ethylene as Quality Index for Bundled Carrots During Postharvest

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Summary

Hand harvested carrots were kept with and without foliage for four days in a plant cuvette and their ethylene production, endogenous ethylene concentration, and corresponding 6-methoxymellein content were measured. Ethylene was analysed using photoacoustic spectroscopy. Ethylene emissions during postharvest were higher from carrots with foliage than without foliage. A varying light period during storage showed no effect on the ethylene emission rate. Emission increased during the first 10 hours for carrots without foliage but remained constant for carrots with foliage. Ethylene concentration in intercellular air space increased in carrots stored with and without foliage. Despite this, the concentration of 6-methoxymellein increased only during 4 days postharvest period when carrots were kept with leaves. However, for all treatments the final 6-methoxymellein-concentration remained below the tasting threshold. Photoacoustic spectroscopy has been shown to be a very sensitive technique to detect ethylene during product storage.

Zusammenfassung

Kontinuierliche Messung von Ethylen als Qualitätsparameter für Bundmöhren in der Nachernte. Bundmöhren, d. h. Möhren die mit Laub vermarktet werden, produzieren nur sehr geringe Mengen Ethylen. Möhren sind aber auch dafür bekannt, dass sie bereits auf geringe Ethylenkonzentrationen mit der Bildung von Bitterstoffen reagieren. Eine Bestimmung genauer Ethylenproduktionsraten ist daher für die Entwicklung von Qualitätsmanagementsystemen in der Nachernte von Gemüsepflanzen unabdingbar. Handgeerntete Möhren mit und ohne Laub wurden vier Tage in einer Pflanzenküvette unter definierten Klimabedingungen gelagert und die Ethylenabgabe sowie endogene Ethylenkonzentration mittels photoakustischer Spektroskopie bestimmt. Es zeigte sich, dass die Ethylenemission in Möhren mit Laub höher war als in Möhren ohne Laub. Unterschiedliche Lichtregime hatten dabei keinen Einfluss auf die Ethylenproduktion. Nach dem Entfernen des Laubes stieg die Ethylenproduktion für wenige Stunden wundinduziert an. Ein Anstieg endogener Ethylenkonzentration war für Möhren mit und ohne Laub während der viertägigen Aufbewahrung zu verzeichnen. Die Konzentration des Bitterstoffs 6-Methoxymellein stieg hingegen nur in Möhren mit Laub an. In allen Versuchen blieb die 6-Methoxymellein-Konzentration unter der geschmacklichen Nachweisgrenze. Das photoakustische Nachweisverfahren erwies sich als geeignet für den Ethylennachweis in Pflanzen mit sehr geringer Ethylenproduktion.

Key words. 6-methoxymellein – *Daucus carota* – postharvest quality

Introduction

Despite low rates of ethylene production, carrots are very sensitive to changes in C_2H_4-concentration. It is well established, that ethylene plays an important role in plant response to stress (KEEFE et al. 1990; NAKANO et al. 2003). One adverse effect of ethylene exposure is the production of the bitter tasting compound 3-methyl-6-methoxy-8-hydroxy-3,4-dihydroisocumarin (6-methoxymellein) in carrots (FAN et al. 2000; SELJASEN et al. 2000). This bitter taste is undesirable to the consumer and therefore lowers product's quality. Identification of exact ethylene production rates during postharvest is important to predict quality changes in carrots.

In some countries of the European Union, carrots with leaves are bundled and sold in increasing numbers for the fresh-market. The foliage enables consumers to judge the product freshness. Carrots with foliage are subject to high transpiratory water losses, resulting in decreased water potential and cell wall carbohydrates (ULRICHS et al. 1998). Low water potential is also known to increase ethylene production (LAFUENTE et al. 1989; HYODO 1991; SELJASEN et al. 2001a). Still it is

not clear, if carrots stored with foliage produce more ethylene than when stored without foliage. There is evidence that ethylene has an effect on the production of 6-methoxymellein (6-MM) during postharvest (KUROSAKI 1994). It remains unclear if the ethylene production rate in carrots kept with leaves is high enough to alter taste by the production of 6-MM.

The aim of our study was to investigate the effect of foliage on endogenous and exogenous ethylene in carrots. We observed ethylene-production under conditions simulating commercial handling of carrots held with their foliage. Ethylene production was quantified using photoacoustic spectroscopy (PAS), which is known to be a very sensitive tool for trace-gas detection. Ethylene production rates were compared with the 6-MM content in carrots with and without leaves.

Materials and Methods

Plant material

Carrots (*Daucus carota* ssp. *sativus* (Hoffm.)), cultivar 'Nanthya' (F_1-hybrid), were grown in sandy soil at the experimental station of the Humboldt University in Berlin. Cultivation was conducted as recommended and described by BUNDESSORTENAMT (1995). Carrots were carefully harvested by hand to reduce mechanical damage. To minimize age effects on ethylene production we always used the same criteria for harvesting. Harvested carrots had a maximum root diameter of 25±2 mm and a root weight of 70±10 g, according to the EU quality norms for bundled carrots (BICKELMANN 2001). For ethylene determination, carrots were carefully washed under cold water and then transported in polystyrene cooling boxes under water saturated air conditions to the Institute of Applied Physics at the Rheinische-Friedrich-Wilhelms University in Bonn. Time from harvesting to the first measurement of ethylene production did not exceed 24 hours. Carrots were defoliated in experiments with carrots without foliage prior to storage but after the transport from Berlin to Bonn.

Experimental setup of the ethylene detection

For the determination of ethylene emission from carrots with and without foliage, single plants were placed under controlled conditions onto a grid inside a 500 litre plant cuvette, about 2 cm from each other. The cuvette was made of chemically inert materials such as glass, teflon, and stainless steel (Fig. 1).

For gas exchange measurements in a closed cuvette system it is imperative to closely control air ventilation inside the cuvette to obtain representative measurements of a single gas fraction inside the cuvette. Strong air ventilation can increase both the rate of transpiration and respiration of measured products. Therefore, air ventilation inside the cuvette was rather low. Inside the cuvette, ambient climatic parameters were set to values common for postharvest storage of the product (temperature 18 °C and 90 % relative humidity), and monitored continuously during the experiment. Ethylene measurements were performed over a 90 hours period to study a realistic strain of the carrots. The 90 hours period is an estimate of the duration carrots with foliage are in a market before being sold.

Hydrocarbons in the ambient air were removed from the cuvette by flushing the air in a closed cycle over a platinum catalyst (400 °C). Only after the air passes over the catalyst did we begin data acquisition. Oxygen and carbon dioxide concentrations were continuously monitored during the experiment with an URAS and a MAGNOS (both Hartmann & Braun, Hamburg, Germany). From a bypass of the closed gas cycle, two litres per hour were extracted for ethylene measurement and returned to the plant cuvette (Fig. 1). Carbon dioxide

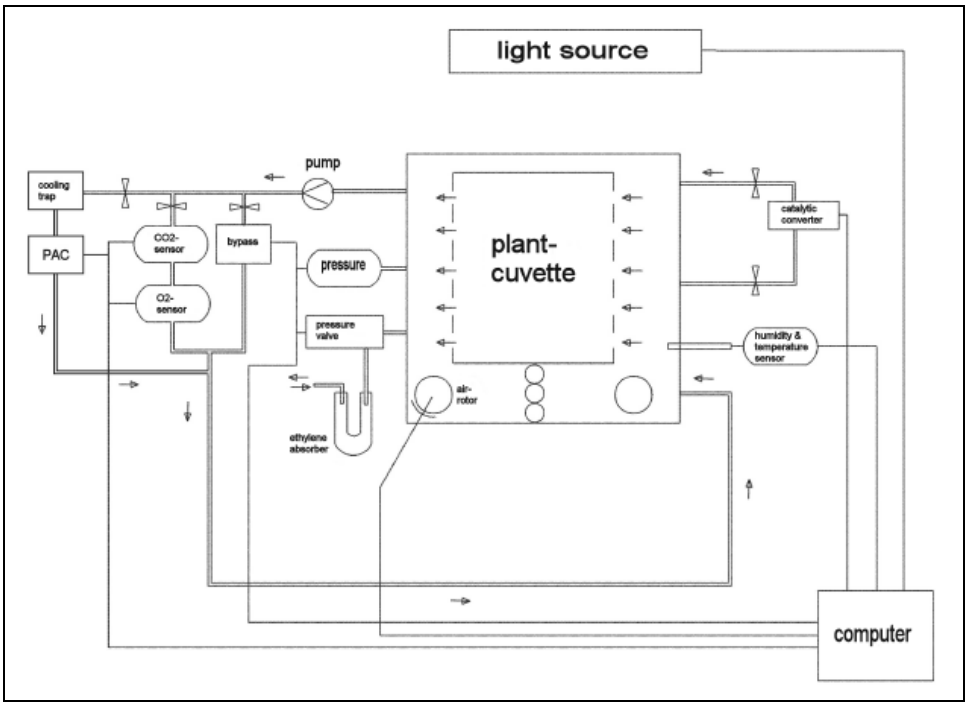


Fig. 1. Experimental design for the photoacoustic ethylene determination (PAC = photoacoustic cell).

and water would affect the accuracy of ethylene measured in the photoacoustic cell (PAC): these were removed quantitatively from the gas sample by passing through a cooling trap using liquid nitrogen (Fig. 1). The vapour pressure of a substance is much more important for a trace gas measurement at the part per trillion level than the boiling point. As long as the concentration of the substance (ethylene) is below the vapour pressure the substance is not trapped. Above the vapour pressure the substance is trapped and only the corresponding concentration of the vapour pressure passes the trap.

The experiment was repeated with a 12 hour interchanging light-darkness regime to determine if photosynthesis influences ethylene emission. For the light regime we used 400 Watt Son-T Agro bulbs from Phillips, Germany. The light intensity per bulb was 100–150 µmol m^{-2} s^{-1}. Light was measured using a CIRAS porometre from PP Systems, Hitching, UK.

Determination of ethylene concentration in intercellular gas

Following the method described by SALTVEIT (1982), a 10-liter desiccator was filled with eight litres of ammonium sulfate solution (20 %) (Fig. 2). The ammonium sulfate reduces the solubility of carbon dioxide in the solution. The ammonium sulfate solution was degased for 10–15 minutes with a negative pressure of 400–420 Pa by using a vacuum pump. After pressure equalized, the desiccator was opened and the carrots carefully added to the solution under an inverted funnel. The funnel was positioned with the opening towards the top and closed at the tip with a gas-tight septum. It was important that air did not accumulate in the funnel. Then, the desiccator was closed and the pressure reduced to minus 400–420 Pa for three minutes.

After release of the vacuum, gas samples were taken with a syringe through the funnel septum and injected into the continuous flow of clean air (oxidized by the catalyst) and measured in a PAC. Ethylene amounts were quantified by integration of the measured graph relative to the volume of carrots. Concentration calculation included product volume and quantity of endogenous gas. No significant changes were found in measured ethylene concentration after three minutes of vacuum up to periods of four hours (data not shown).

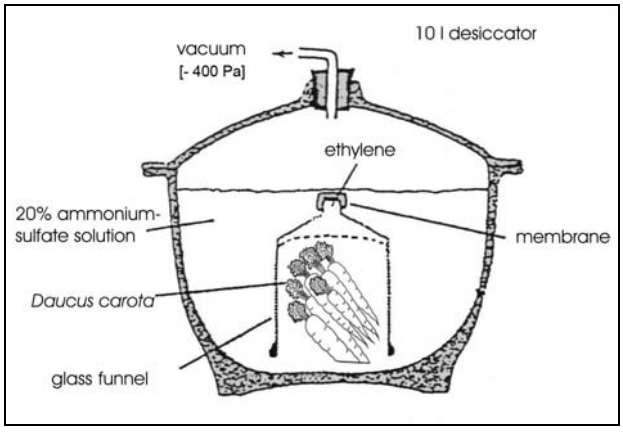


Fig. 2. Experimental design for the extraction of endogenous ethylene.

Isocumarin analyses

Carrots were diced before and after storage and shock-frozen in liquid nitrogen. Samples were placed on dry ice and sent to the Norwegian Food Research Institute in Osloveien, Norway for isocumarin analysis. The extraction and quantification of 6-methoxymellein was done as described by SELJASEN et al. (2000).

Statistics

Endogenous ethylene concentration and 6-MM content were established in full-factorial experiments with five replications of 10 carrots each. Data were analysed by ANOVA and Tukey's pairwise comparison test was used for differences between treatments.

Results

The ethylene concentration increased linearly during the experimental period of 90 hours in the plant chamber containing carrots with foliage (Fig. 3). The production rate during this time was 0.068 µl h^{-1} kg^{-1} fresh weight, while the fresh weight decreased from

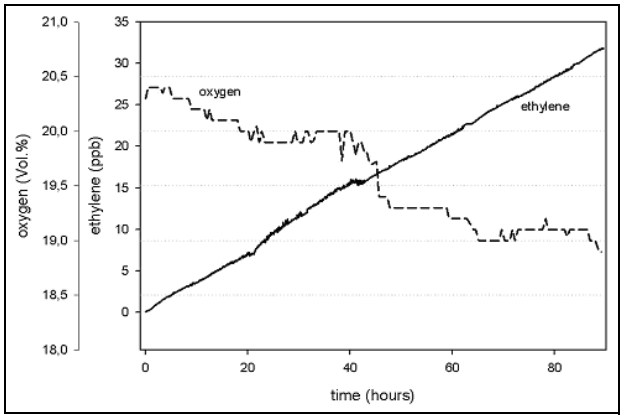


Fig. 3. Accumulation of ethylene from carrots with foliage and effect on oxygen content in a 500 l plant cuvette during post-harvest storage at 18°C and 90% rh

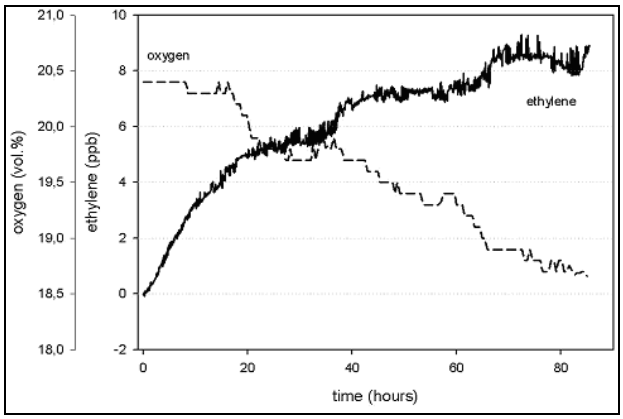


Fig. 4. Accumulation of ethylene from carrots without foliage and effect on oxygen content in a 500 l plant cuvette during post-harvest storage at 18°C and 90% rh

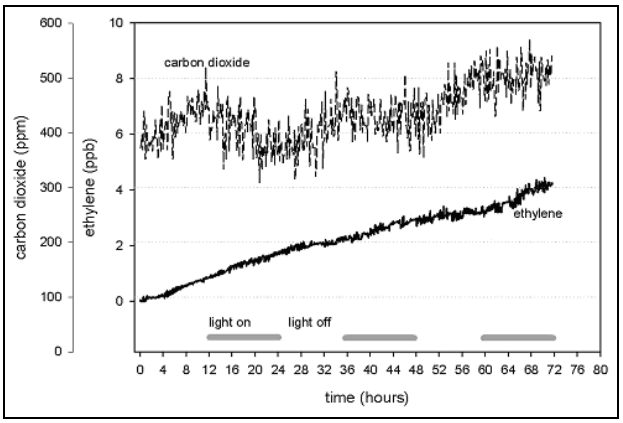


Fig. 5. Accumulation of ethylene from carrots with foliage and effect on oxygen content in a 500 l plant cuvette influenced by light during post-harvest storage at 18°C and 87% rh

2.83 to 2.06 kg. At the same time, the oxygen concentration decreased continuously in the chamber to a final concentration of 19 % (Fig. 3). In contrast, the ethylene concentration inside the chamber with carrots without foliage increased logarithmically (Fig. 4). Ethylene production rate was highest during the first 10 hours of the experiment (0.040 µl h^{-1} kg^{-1} fresh weight) but declined to 0.022 µl h^{-1} kg^{-1} after 10 hours. Therefore, carrots with foliage produced more ethylene than those, which had foliage removed prior to storage.

In a second experiment carrots with foliage were held under a light regime of alternating 12 h light and 12 h darkness-periods. The ethylene production rate (0.069 µl h^{-1} kg^{-1}) was similar to that observed in the experiment without illumination. The ethylene concentration at the end of the experiment inside the cuvette was about 4 ppb (Fig. 5). During the light period, carbon dioxide concentration in the chamber decreased as a result of photosynthesis and increased again during the dark period. The amplitude of the carbon dioxide curve reflected the effects of respiration and photosynthesis and decreased as plants senescenced.

The intercellular ethylene gas concentration of carrot roots increased during 4 days of storage in both carrots, stored with and without foliage. The final concentration was significantly higher when carrots were stored with still attached leaves (4.5 ppm) than without leaves (4 ppm) ($F_{2,12}$=107.7; P<0.001). The corresponding 6-methoxymellein content was also significantly higher in carrots stored with foliage than in the untreated control, but not in carrots kept without foliage ($F_{2,12}$=704.6; P<0.001) (Table 1).

Discussion

Carrots kept four days with and without foliage were still physiologically active and emitted ethylene continuously. Both, the ethylene released and the intercellular ethylene concentration was higher in carrots with foliage than in carrots without foliage. Ethylene emission by carrots without foliage declined after 10 hours. The increased ethylene emission is most likely a result of removing foliage. Wound induced ethylene production, has been described by many authors (KATO et al. 2000). Nevertheless, ethylene emission was much lower during the whole storage period when foliage was removed. We cannot rule out completely the effect of plant age on ethylene production, as there was a 4–5 day difference in harvest dates for plants with and without foliage. Nevertheless, we tried to minimize that effect by using identical harvest criteria. CO_2 produced during respiration can slow the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene (ABELES et al. 1992). However, the oxygen level in the chamber was never below 19.5 Vol.% and therefore the CO_2 concentration did not reach concentrations sufficient to limit ethylene production. During light periods, the carbon dioxide concentration in the cuvette decreased but increased during darkness as a result of photosynthesis and respiration. The ethylene emission was not influenced by photoperiod. In contrast, BUCHER and PILET (1981) reported high ethylene production during photosynthesis in peas and maize.

It must be noted that ethylene production rate and endogenous concentration depend on the physiological stage of the product and on stress conditions that occur during handling. Additionally, there are most likely differences among cultivars as shown by DEVRIES et al. (1996) for different fruits and vegetables. Therefore, the data presented are an indicative measure of gas production and emission of these particular batches of carrots.

Low ethylene concentrations may induce the production of the isocumarin 6-methoxymellein in carrots (LEJA and MARECZEK 1995). Isocumarins are responsible for the bitter taste in carrots (SHATTUCK et al. 1988). Carrots exposed to 1 ppm ethylene in air for 21 days showed a gradual increase in 6-MM content, whereas carrots exposed to ethylene-free air showed at low level of 6-MM (SELJASEN et al. 2001b). Like other phytoalexins, 6-MM is produced in nonphotosynthetically active tissue as a result of stress (LAFUENTE et al. 1996). However, the complete pathway for 6-MM production remains unknown. Our results indicate that there are other elictors of 6-MM production besides ethylene, because its concentration differed between carrots with foliage compared to those without foliage. That is, the concentration of endogenous ethylene for both treatments was significantly higher than the control: however, 6-MM content for carrots

Table 1. Endogenous ethylene concentration and 6-methoxymellein-content in carrot roots.

treatment (4 days at 18 °C, 88 % RH)	C_2H_4 concentration* (ppm)	6-MM content (µg g^{-1} fresh weight)
untreated	2.879[a]	0.035[a]
roots stored 4 days without leaves	4.083[b]	0.038[a]
roots stored 4 days with leaves	4.527[c]	0.358[b]

(letters indicate pairwise comparison with Tukey's test within columns, p<0.05 for 6-MM content; * ethylene concentration was measured 24 hours after harvesting, p<0.01)

without foliage was similar to the control, but 10 times higher than the control for carrots with foliage. We believe the high level of 6-MM in carrots stored with foliage occurs from water loss through transpiration leading to an increase in concentration. YOSHINO et al. (1993) and KAWABATA et al. (1995) have reported that a 6-MM concentration of 60 µl ml^{-1} is detectable by the human tongue and describe carrots as bitter tasting when a concentration of 100 µl ml^{-1} solution is reached. Based on their data the 6-MM content in our experiments was below the threshold level for bitter taste.

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