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Development of a Chromatography System for Simultaneous Measurement of Atmospheric Gas Components: Use in Measurement of Oxygen, Carbon Dioxide, Nitrogen, Argon, and Ethylene within Individual Fruit and in the Storage Atmosphere

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A gas chromatographic (GC) system was developed to determine oxygen (O_2), argon (Ar), carbon dioxide (CO_2), nitrogen (N_2), and ethylene simultaneously. The system consists of a GC equipped with one thermal conductive detector (TCD), one flame ionization detector (FID), one split/splitless inlet, and one packed column inlet. The system uses porous polymer and molecular sieve capillary columns to perform the separation of the gaseous species. A gas sample is introduced by a two-position valve fitted with two sample loops, to two flow paths within the GC. One path goes directly from the packed inlet, through a styrene divinylbenzene column to the FID to detect ethylene. The other goes through the split/splitless inlet, which is fitted with two separate columns, one to separate and detect CO_2, and another to separate Ar, O_2, and N_2 before detection by the TCD. The subsystem for the Ar, O_2, and N_2 separation contains a protection column (pre-column) and a separation column (molecular sieve) separated by a two-position valve. The valve is switched before the water and CO_2 elute from the pre-column and go into the molecular sieve column. Water and CO_2 would cause deactivation of the molecular sieve column. The entire run takes less than 12.8 min. This system has been used for the analysis of internal gas composition in fruit coated with different waxes, monitoring of gas combination in controlled atmosphere storage rooms, and other applications. It is a key tool to conduct individual fruit physiology and quality research, and for finding direct correlation between internal gases and fruit physiological metabolism and quality.

Oxygen and CO_2 determination is a must for controlled atmosphere (CA) storage, modified atmosphere packaging (MAP), and edible coating research and commercial maintenance of fruits and vegetables (Baldwin and Hagenmaier, 2011; Beaudry, 1999; Forney et al., 2009; Mir and Beaudry, 2004). Low O_2 and elevated CO_2 concentration in the atmosphere benefits some horticultural crops by extending storage life and maintaining a better appearance, flavor, and nutrient quality (Bai et al., 2003; Baldwin and Hagenmaier, 2011; Forney et al., 2009). Improper low O_2 and/or high CO_2 atmosphere may cause various plant physiological disorders of horticultural crops (Bai et al., 2009). Typical disorders are death of cells on skin or internal tissue, and off-flavor. Beneficial effects of and tolerance to low O_2/high CO_2 levels depend on physiology of horticultural crops. For instance, 2 kPa O_2 + 2 kPa CO_2 CA benefits storage of 'Delicious' and 'Granny Smith'; however, the same CA condition caused internal breakdown of 'Braeburn' apples (Bai et al., 2003).

When O_2 is measured by gas chromatography (GC) and the concentration of O_2 is as low as 1%, Ar can significantly influence the determination of O_2. Argon concentration in regular atmosphere (air) is 0.933% vs. 21% of O_2. In most GC systems, O_2 and Ar share a similar retention time and are not separated. If O_2 is determined at about 1% in a CA storage room, a MAP package, or an internal gas sample from a coated fruit, it might mean that the "1%" is almost all Ar, or the O_2 content is close to 0. Several factors can contribute to a low O_2 level relative to Ar including: O_2 is consumed by respiration but Ar is not; packaging films or coatings having differential permeabilities to different atmospheric gases; and exclusion of O_2 by a N_2 generator, but not Ar (Bai et al., 2003; Hagenmaier, 2003). Therefore, the separation of Ar from O_2 is important especially when O_2 is very low. Hagenmaier (2003) used a CTR III column (Alltech, Deerfield, IL), comprised of two concentric packed stainless-steel tubes, the outer tubing having a ¼-inch outside diameter and packed with an activated molecular sieve packing that separated N_2 and O_2 + Ar. The packing of the inner column was able to separate Ar from other components. Oxygen concentration was calculated by [O_2 + Ar] – [Ar], but this was not quite accurate though, because the response factor of O_2 and Ar might be different.

Ethylene is another important gas for horticultural crops. A low concentration, as low as a few parts per million (ppm) could cause deterioration of ethylene-sensitive crops (Baldwin, 2004). Usually ethylene measurement is simple, accurate, and fast using a GC. However, ethylene is detected by a flame ionization detector (FID), in comparison to CO_2, O_2, and Ar, which are detected by a thermal conductive detector (TCD). These gases generally are

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measured by two GCs or one GC equipped with both detectors, by two separated sample injections (Bai et al., 2003).

When conducting edible coating research in fruit, such as apples, it is desirable to measure the internal O_2, CO_2, and ethylene simultaneously. Thus, the change of individual gas components, as well as the correlations among gas components can be analyzed. Usually, the internal gas collected from a fruit is only enough for one GC sample injection. When using a group of fruit for ethylene measurement, another group for O_2 and CO_2 measurement, and the third group for Ar measurement, the data will not be strictly suitable for statistical correlations, although many studies proceed to incorrectly correlate these data even though the gas analysis is not on the same group of fruit.

In this research, we developed a GC system, which determines O_2, CO_2, N_2, Ar, and ethylene simultaneously, allowing analyses of all these gases in one sample. Using this system we measured internal gas components in carnauba and shellac-coated 'Braeburn' apples.

Materials and Methods

GC configuration and operating scheme. A Hewlett-Packard 5890 II (Avondale, PA) GC equipped with a TCD and a FID was used. A two-position 10-port valve (V1) (VICI Valco Instruments, Houston, TX) fitted with two sample loops was used to introduce the gas sample to the two flow paths of the GC, one side through the packed inlet and the other through the split inlet (Fig. 1). On the packed inlet side with a 122 cm × 0.16 cm i.d. loop, ethylene is separated by Column #1 (C1), a 30 m × 0.53 mm i.d. porous polymer (styrene divinylbenzene, AT-Q, Alltech, Deerfield, IL), and detected by the FID (Fig. 1 and Table 1).

On the split inlet side with a 18 cm × 0.16 cm i.d. loop, two capillary columns were fitted to the inlet (Fig. 1). Column #2 (C2), a 25 m × 0.53 mm i.d. porous layer open tubular (PLOT) polystyrene-divinylbenzene (HP PLOT-Q, Hewlett-Packard) was used to separate CO_2 from other gas constituents (Fig. 1 and Table 1). The other column fitted in the split inlet was Column #3 (C3). It was a short, 5 m HP PLOT-Q same as C_2, guard column. The purpose to use C3 is to work together with Valve #2 (V2, four-port, VICI Valco Instruments) to vent water and CO_2 prior to elution of O_2, Ar, and N_2 and thereby prevent deactivation of Column #4 (C4), which was used to separate O_2, Ar, and N_2. C4 is a 30 m × 0.53 mm i.d. molecular sieve 5A column (HP MS-5a, Hewlett-Packard). V2 was used to place C3 and C4 in-line and out-of-line with each other, for different aspects of the separation process (Fig. 1 and Table 1). CO_2, O_2, Ar, and N_2 were detected by the TCD.

GC conditions. Five milliliters of sample gas was injected into

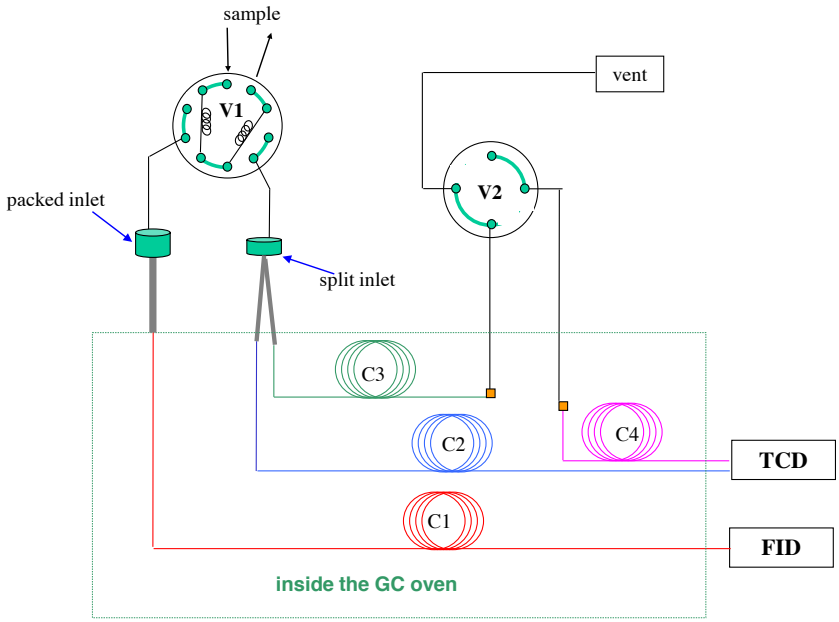

Fig. 1. GC configuration and operation scheme. C1–C4: columns; V1 and V2: valves. See Tables 1 and 2 for model and technical parameters of the major components. TCD: thermal conductivity detector. FID: flame ionization detector.

Table 1. Valves and columns used in the GC system (see Fig. 1 for GC configuration and operation scheme).

Part	Abbreviation	Model/detail		
Valve #1	V1	10-port Valco used for simultaneous injection into two paths		
—Loop to packed inlet		—122 cm × 0.16 cm i.d. (2.45 mL) stainless steel tubing		
—Loop to split inlet		—18 cm × 0.16 cm i.d. (0.36 mL) stainless steel tubing		
Valve #2	V2	4-port Valco stream switching		
Column #1	C1	Alltech AT-Q	30 m × 0.53 mm i.d.	20-µm coating
Column #2	C2	HP PLOT-Q	5 m × 0.53 mm i.d.	20-µm coating
Column #3	C3	HP PLOT-Q	25 m × 0.53 mm i.d.	20-µm coating
Column #4	C4	HP MS-5a	30 m × 0.53 mm i.d.	50-µm coating
Flow controller #1	Fc#1	0–20 mL/min Porter VCD-1000		

Table 2. GC condition - typical analysis.

Temperatures	
Oven	30 °C (3.5 min) then at 15 °C·min^{-1} to final 140 °C (hold 2 min)
TCD	175 °C
FID	225 °C
Packed inlet	175 °C
Split	175 °C
Flow rates	
C1	10.0 mL·min^{-1}
C2	6.5 mL·min^{-1}
C3 and C4	6.5 mL·min^{-1}
TCD reference	20.0 mL·min^{-1}
TCD make-up	3.0 mL·min^{-1}
FID H_2	30 mL·min^{-1}
FID Air	350 mL·min^{-1}
FID make-up	23 mL·min^{-1}

the GC. The flow rate for C1 was 10 mL·min^{-1}, and C2–C4 was at 6.5 mL·min^{-1}. The initial oven temperature was 40 °C, remained for 3.5 min, and then increased to 140 °C at a rate of 15 °C min^{-1}, where the temperature was held constant for an additional 2 min. The GC peaks for each component were quantified using standard curves as determined by known concentrations of gas standards. Table 2 shows detail for the GC conditions.

Analysis of internal gas in apple fruit and storage atmosphere gas in a CA room. Preparation for coating treatment: 'Braeburn' apples were harvested at commercial maturity from a commercial orchard located at Hood River, OR. They were randomly packed into wooden boxes with perforated polyethylene liners. After storage at 2 °C for 2 months, fruit were transferred to 20 °C and held for 24 h, prior to applying coatings.

The following two experimental coatings were used: shellac coating containing 19.3% shellac (R52, Mantrose Haeuser, Atttleboro, MA), 1.2% oleic acid (Emersol 6321, Henkel, Cincinnati, OH), 4.6% morpholine (Sigma Aldrich, St. Louis, MO), and the total solids was 25.1%; and carnauba coating containing 19.0% carnauba (No. 3, light, Strahl & Pitsch, West Babylon, NY), 3.1% oleic acid, 2.4% morpholine, and the total solids was 24.4%.

For each coating, 30 fruit were coated using latex-gloved hands with 1.0 mL coating per fruit. Instead of coating, water was used for control fruit. A conveyer-fed hot-air drying tunnel in a pilot-plant scale packing line (Rush & Associates, Wenatchee, WA) was used to dry fruit at 50 °C for 4 min. Coated fruit were stored at 20 °C for 7 d prior to analyzing internal gas combination.

Sampling of internal gas. Samples of internal gas were obtained from the seed cavity of fruit using a 30-mL syringe under submerged condition. The internal gas volume averaged 7.26 mL and ranged from 5.3 to 12.1 mL. Preparation of gas samples from CA storage room: 'Anjou' pear fruit harvested at commercial maturity from a pear block located at Mid-Columbia Agricultural Research and Extension Center, Hood River, OR, were packed in 180 wooden boxes (about 3,600 kg) with perforated polyethylene liners and loaded in a pre-cooled (1 °C) storage room. Hydrated lime (1 kg per packed box) in perforated paper bags was used to absorb respiratory CO_2 evolved from the fruit prior to sealing an air-tight room. Then the room was cooled to –1 °C within 2 d, and flushed with N_2 to an O_2 concentration of 0.7% within 4 d. After 3 months, the O_2 concentration had risen to 1.5% combined with <0.5% CO_2 (Bai et al., 2009). The gas combination in the storage room was determined twice per week for 4 months. A gas sample was taken from the center of the storage room through polyethylene tubing (10-mm diameter). Three samples were measured at each sampling time to represent three replicates.

Statistical analysis. SAS Version 9.1 (SAS Institute, Cary, NC) was used for analysis of instrumental analytical data. Each gas component with 30 replicates was analyzed using analysis of variance (PROC ANOVA). The treatment means were separated at the 0.05 significance level by the least squares means test (LSD). For linear regression, PROC REG was used.

Results and Discussion

Separation and detectability of gas components. Ethylene was completely separated from N_2 under FID, and the detection limit was as low as 0.1 ppm (Table 3). The CO_2 peak was on the tail of the $N_2 + O_2$ peak, and easily detectable (Fig. 2). The detection limit was as low as 100 ppm (Table 3). On the other hand, the retention times of Ar and O_2 were very close, with a 5.4 s of difference (Table 3 and Fig. 2). When the O_2 concentration was higher than 5%, the detectable Ar level rose to >0.5% (Table 3 and Fig. 2). Fortunately, measurement of Ar is important only when the O_2 concentration is low, such as <2%, when Ar and O_2 are separated completely (Table 3 and Fig. 2). If Ar was higher than 5%, the detectability of O_2 also dropped. However, under natural air conditions, Ar is about 1% or lower.

Sample size and run time. Under the GC conditions in this experiment, the minimum sample size was 3 mL, including 2.45 mL for the packed inlet, and 0.36 mL for the split inlet (Table 1). Such a high sample volume, 2.45 mL, was to improve ethylene detection. A decrease in the injection volume drops detectability, therefore, an increase of injection volume would improve detection.

The total run time was 12.8 min. The oven temperature program was: initial temperature 30 °C for 3.5 min, then increased to 140 °C at a rate of 15 °C·min^{-1}, and finally remained at 140 °C for 3 min. Prior to this set-up, our GC efforts measured O_2, CO_2, ethylene and/or Ar individually with multiple GCs, multiple injections (multiple sub-samples), and each measurement needing up to 3–4 min, which was time consuming and labor intensive (Bai et al., 2003, 1990; Hagenmaier, 2003). Further, the new method also avoids the potential water deactivation of

Table 3. Retention time and lowest detectable concentration of CO_2, ethylene, Ar, O_2, and N_2 analyzed by a GC.[z]

Compound	Retention time (min)	Lowest detectable concn	Remarks
CO_2	2.12	100 ppm	
Ethylene	2.85	0.1 ppm	
Ar	3.72	0.1% to 0.5%	As O_2 concentration increases in a mixed gas system, detectability of Ar decreases, and vice versa.
O_2	3.81	0.1% to 0.5%	
N_2	5.48	1%	

[z]GC conditions are shown in Tables 1 and 2.

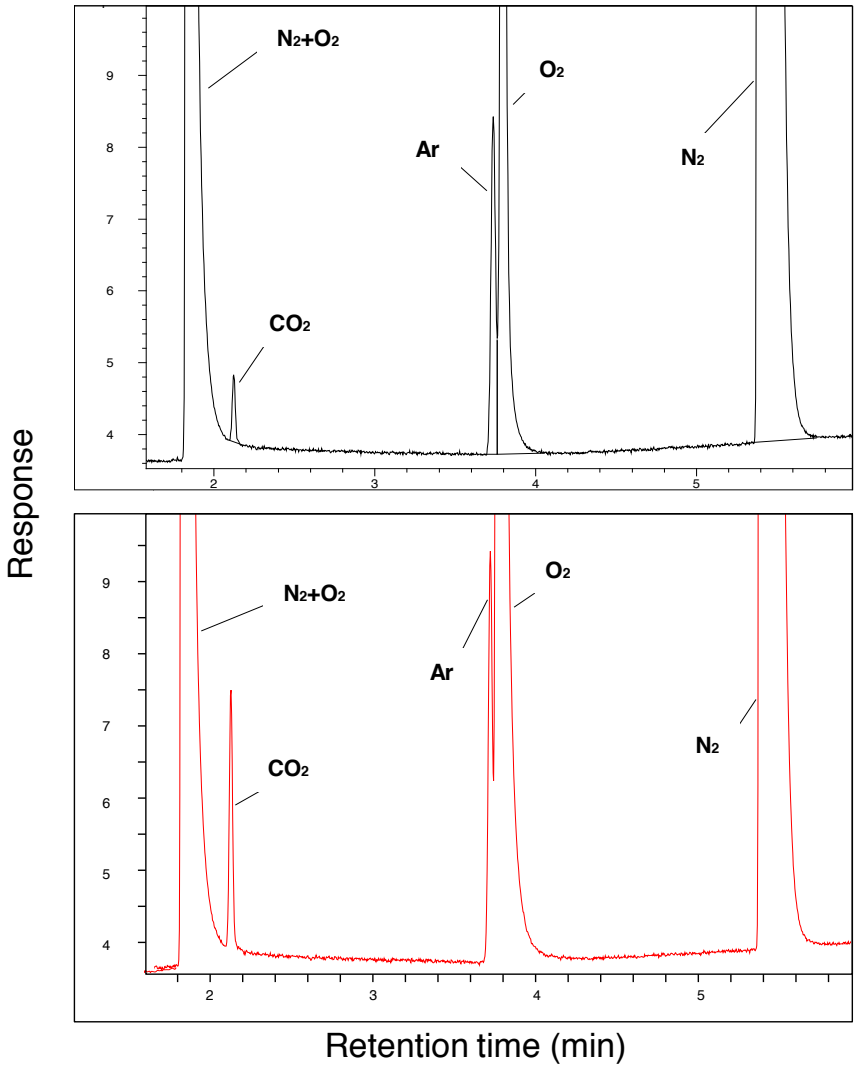

Fig. 2. The separation as seen on the TCD. The chromatogram on top shows 0.1% CO_2, 1% Ar, 5% O_2 and 97.9% N_2. The chromatogram on the bottom shows 0.4% CO_2, 1% Ar, 17% O_2 and 81.6% N_2.

the molecular sieve column. The old methods required baking the column for a few hours to reactivate it after a few injections of high moisture samples (Hagenmaier, 2003).

Determination of internal gas in coated apples. Shellac coating caused extremely low O_2 in fruit samples. The average internal O_2 concentration in shellac-coated fruit was 1.2% and there were 23% of the fruits containing undetectable levels of O_2 (Fig. 3). This coating caused marked increases of internal CO_2, and also ethylene concentration (Fig. 3). Under low O_2 and/or elevated CO_2, the ethylene production usually is low; however, ethylene builds up in the fruit due to low permeability of the coating to ethylene (Bai et al., 2002). Such a low O_2 and elevated CO_2 caused off-flavor. Conversely, carnauba coating created a modified internal atmosphere in the fruit of 7.2% O_2 + 7.1% CO_2 (Fig. 3). This extended the storage time of the fruit without causing off-flavor, in comparison to the non-coated control, which had a gas combination not much different from the ambient atmosphere, but had a short storage life.

If a GC is not equipped with cryogenic oven cooling, Ar and O_2 elute close together, which thwarts the accurate measurement of O_2. Therefore, the relationship between internal O_2 and Ar concentration was analyzed. The results showed that there was no significant linear regression between the two components (r^2 = 0.423, Fig. 4).

On the other hand, there was a significant linear regression between N_2 and Ar concentration measured on coated apples and in a CA storage room containing stored 'Anjou' pears (Fig. 5). Both N_2 and Ar are considered as "inert" gases and neither are involved in respiration or other chemical or biochemical reactions in fruit. In non-coated fruit, both gases exchange between inside and outside of the fruit with little resistance (Bai and Plotto, 2011; Hagenmaier, 2003). For coated fruit, the resistance to gas exchange caused by the coating, as well as consumption of O_2 and production of CO_2 caused by respiration of fruit tissue resulted in the creation of an internal atmosphere with low O_2 and high CO_2. The internal CO_2 + O_2 concentration is usually lower than 21%. However, when respiration is very high and the coating permeability is very low, the sum of these concentrations can be higher than 21% (Bai et al., 2003). Steady-state internal O_2 and CO_2 levels may be attained within 5 to 6 h after coating (Bai and Plotto, 2011; Petracek et al., 1999) at a given storage temperature, and the internal gas levels often change little once steady state

Average gas concentration in fruit.

Coating	CO_2	O_2	Ethylene (ppm)
Control	2.5 ± 0.8 c	18.3 ± 1.0 a	193 ± 139 c
Carnauba	7.1 ± 3.2 b	7.2 ± 2.4 b	534 ± 136 b
Shellac	13.7 ± 3.4 a	1.2 ± 2.5 c	735 ± 155 a

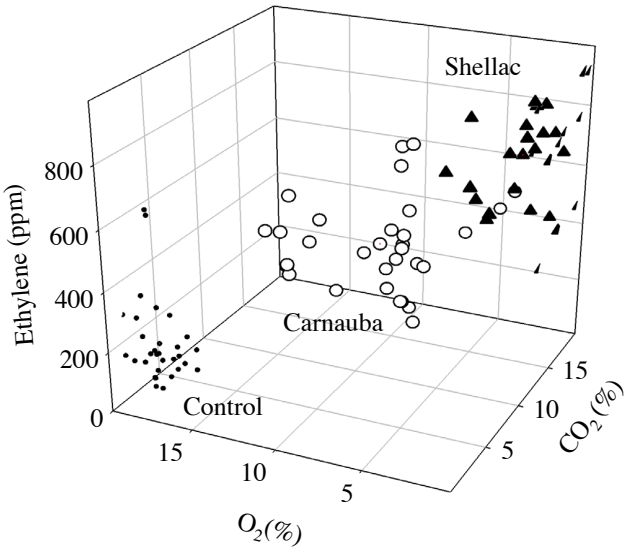

Fig. 3. Internal CO_2, O_2, and ethylene concentrations of 'Braeburn' apples coated with carnauba, shellac, and non-coated controls. Smashed triangle marks represent shellac coated fruit when the O_2 concentration is equal to zero.

is attained in citrus fruit (Hasegawa and Iba, 1980) if there is no change in storage temperature. However, the change in internal gas levels can be remarkable when fruit are near the climacteric rise of respiration, especially for newly harvested 'Braeburn' fruit (Bai et al., 2003). Fruit used in this experiment did not show a significant change of respiration rate after coating and storage at 20 °C for 7 d, likely because they were stored for 2 months prior to being used for this experiment.

The internal gas data showed that 1) the internal O_2, CO_2, N_2, and Ar summed to 99.5% of total gas, the rest is assumed to be water vapor and minor components (Figs. 3–5); 2) there were linear regressions between internal N_2 and Ar with regression equations (Fig. 5) of:

In apple fruits: $[Ar\%] = 0.01048\ [N_2\%] + 0.0849$ (Equation 1)

In the CA room: $[Ar\%] = 0.01169\ [N_2\%] - 0.01811$ (Equation 2)

By combining the data from apple internal gas and the CA room, a general regression equation is:

$[Ar\%] = 0.01126\ [N_2\%] + 0.02139$ (Equation 3)

The results indicate those equations may serve for prediction of Ar concentration.

Determination of gas combination in CA storage room. The combination of gases in the commercial CA storage rooms usually are monitored by an electrochemical sensor. Ar does not influence the reading of O_2. Other widely used oxygen analyzers, such as paramagnetic, polarographic, or zirconium oxide sensors are also not influenced by Ar in the gas constitutes (Meriläinen, 1989). However, in a postharvest research laboratory, a GC-TCD is more likely to be used. Some of the obvious reasons are because it requires a smaller volume of gas sample, provides more accuracy, and is suitable to a much wider range of concentration.

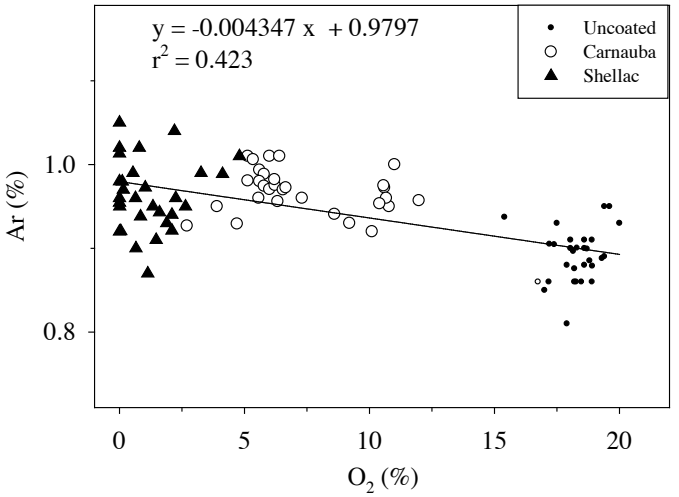

Fig. 4. Relationship between internal O_2 and Ar concentration of 'Braeburn' apples coated with carnauba, shellac, and non-coated controls.

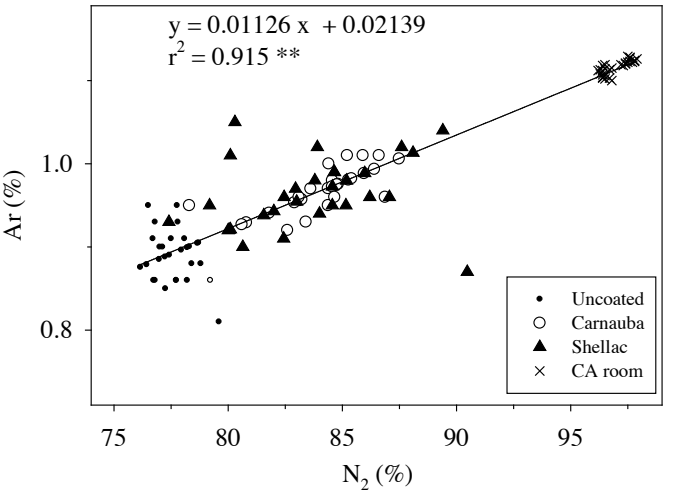

Fig. 5. Relationship between N_2 and Ar concentrations in the internal 'Braeburn' apples and in a CA room containing 'Anjou' pears. Fruit were coated with carnauba and shellac. Non-coated controls were used.

In the first 3 months of 'Anjou' pear storage, the oxygen, CO_2, N_2, and Ar concentrations were 0.70 ± 0.04, 0.06 ± 0.02, 96.58 ± 0.35, and 1.12 ± 0.08%, respectively. After 3 months, the oxygen concentration was regulated to be 1.50 ± 0.07%, CO_2 0.38 ± 0.16%, N_2 96.49 ± 0.27%, and Ar 1.11 ± 0.08%, respectively. The GC clearly separated the above mentioned compounds. Ar and N_2 concentration showed linear regression (Fig. 5).

Conclusion

A GC methodology was developed to simultaneously determine O_2, CO_2, ethylene, N_2, and Ar concentrations in a fruit or in a CA storage room. The system requires a minimum gas sample of 3 mL, and has a run time of 12.8 min. The system was suc-

cessfully used to determine internal gas concentration of coated apples and gas combination in a CA storage room. It is especially useful for accurately measuring O_2 concentration at anaerobic storage conditions.

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