

A Fast Gas Chromatographic Method for the Separation of Nitrogen, Oxygen, Carbon dioxide and Argon and Its Application to In-package Modified Atmosphere

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A gas chromatographic method was developed for the rapid separation of headspace gases in film-package of fresh produce. A balanced set of three columns simultaneously separated four gases including N₂, O₂, CO₂ and Ar. The gas composition was accurately calculated by measuring the ratio of each peak area on the chromatograms and considering the relative response of a thermal conductivity detector to each gas. This method was found to be useful for the accurate determination of O₂ and CO₂ in the modified atmosphere, because Ar proved to be kept in film packages of the produce at a concentration above ca. 0.9% in various cases while Ar overlaps O₂ on the gas chromatograms in conventional gas chromatographic systems.

Keywords : Argon, Oxygen, Carbon dioxide, Fresh produce, Headspace gas, MA packaging, Gas chromatography, Gas composition

INTRODUCTION

Modified atmosphere packaging (MAP) has been studied as a technique to prolong the shelf-life of fresh vegetables and fruits¹⁾²⁾. The technique basically expects self-equilibration of the in-package gas composition, which may provide a suitable atmosphere for controlling excess respiration of the fresh produce during

distribution. Since a variety of technical information including permeability of films, respiration rates in a given O₂/CO₂ atmosphere, respiration quotients, free volume of the package, etc. are required for the proper design of MAP, computer aided simulation is one of the most promising techniques to obtain suitable MAP conditions^{3)~8)}.

For any MAP design, the exact determi-

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nation of the in-package concentrations of N₂, O₂, CO₂ and Ar is necessary, because either anaerobic conditions or the presence of excess CO₂ results in the development of off-odors, and also the higher concentration of O₂ allows for a rapid senescence. Because of the demands for the rapid and simultaneous determination of the in-package gas composition in MAP, gas chromatographical methods have been used. Brenner⁹⁾ described a combination of silica gel and molecular sieve 5A as parallel columns. Karel et al¹⁰⁾ used columns packed with silica gel and a molecular sieve that were connected in series. Recently, Yamashita et al¹¹⁾ used a combination of activated charcoal and a molecular sieve as a parallel column system and calculated the gas composition from the ratio of the peak areas. Aerograph A90 - P3⁶⁾ or Alltech CTR I³⁾¹²⁾ have been also used for analyzing the gas composition, while even in some recent papers¹³⁾¹⁵⁾, the gas analysis procedures are not given in detail. In these columns, Ar is not separated from

O₂ and overlaps O₂ on gas chromatograms. However the presence of Ar in MAP should be considered for establishing the MAP system because in many cases the critical levels of O₂ for providing proper MA conditions are less than 5%.

The objectives of this paper are to develop a gas chromatographic method for simultaneously measuring concentrations (%) of O₂, N₂, CO₂ and Ar in MAP, and to evaluate the gas exchange in MAP of fresh produce on the market or during storage using the method developed.

MATERIALS AND METHODS

1. Vegetables and packaging

Locally grown broccoli was purchased at a wholesale market, packaged in plastic films and stored at 15°C and 90%rh. The characteristics of the films used and the packaging conditions are listed in Tables 1.

Fresh vegetables packaged using flexible

Table 1 Properties of plastic films used for broccoli packaging and the conditions.

Condition	Film type	Film properties at 15 °C, 90 % rh			Initial volume	Broccoli weight	
		Permeability		Thickness			
		(O ₂)	(CO ₂)				
cc/day · m ² · atm	μ	m ²	ml	g			
A	PMP*	23,400	92,200	55	0.124	1036	233
B	LDPE**	7,640	33,900	18	0.124	834	232
C	OPP***	1,460	5,170	20	0.124	1046	239

* Polymethylpentene

** Low density polyethylene

*** Oriented polypropylene

plastic materials were purchased at a local supermarket. The packaging materials were as follows : A stretch film of polyvinyl chloride for shiitake fungus (*Lentinus edodes Sing.*), shimeji fungus (*Pluribus ostreatus Quel.*) and cucumber, low density polyethylene for mung bean sprout (*Vigna radiata Wilcz.*), a mix of shredded vegetables including mung bean sprout, cabbage, carrot and black fungus (*Auricularia auricula-judae Quel.*), and polypropylene for carrot,

2. Gas chromatography

A gas chromatograph (GC - 7A, Shimadzu, Japan) equipped with a thermal conductivity detector (TCD) was used to analyze N₂, O₂, CO₂ and Ar in a gas mixture. Carbon dioxide was separated from the mixture by a stainless steel column (3mm × 0.5m) packed with activated charcoal (mesh : 60~80). Oxygen, N₂ and Ar were separated by a packed column (3mm × 6m) of Gaskuropack 54, (a styrene /divinyl benzene copolymer, mesh : 60~80), while CO₂ was absorbed in the packing material. Both columns were linked in parallel so that simultaneous analysis of the four gases were carried out using a single injection of the gas sample. For controlling the split ratio of the gas flow at a diverging point of the linked column, a packed column (3mm × 4m) of Uniport B (mesh 60~80) was added in series to the activated charcoal column. The total column system is shown in Fig. 1. Three meter column of Uniport B was also connected to TCD as a reference column.

While Uniport B and activated charcoal were controlled at 70°C in the column oven, Gas-

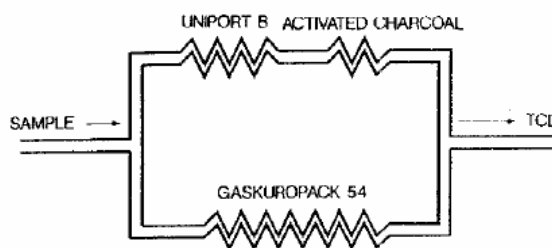


Fig. 1 A schematic diagram of the column system simultaneously separating four gases.

kuropack 54 was separately cooled down to -80°C by soaking the column in dry ice-methanol. Temperatures of the TCD and injection port were adjusted to 90°C. The flow rate of the carrier gas (He) was 60ml/min. Peak areas in a gas chromatogram were measured using a Shimadzu C-RIA integrator. The packing materials for columns were all purchased from GL Science (Tokyo).

3. Gas analysis

About 0.3ml of the headspace gas was periodically sampled from the package and was injected in the gas chromatograph. A small silicone rubber piece (ca.10mm × 10mm) was glued on the surface of the package so that the gas sampling was easily carried out by direct sticking of a syringe needle into the package.

4. Calculation of gas composition

The gas chromatographic profile of a gas mixture including N₂, O₂, CO₂ and Ar is schematically given in Fig. 2. Peak A to B and Peak C to F indicate the gas portions separated by the columns packed with activated charcoal and Gaskuropack 54, respectively.

RESULTS AND DISCUSSION

1. Gas chromatograms

A clear separation of CO₂ from a gas mixture including N₂, O₂, CO₂ and Ar were performed by a single column (3mm × 0.5m) packed with activated charcoal, while O₂, N₂ and Ar were separated from each other on Gaskuropack 54 (3mm×6m) at ~80°C (Fig. 3). These single columns made it possible to separate the gases within a short retention time. However, for determining four gases, two measurements were required and thus careful calibration of an absolute amount of the injected gases was very important for having accurate results. Therefore, in order to simultaneously analyze the four gases, the two columns were linked in multiple connec-

tion. But the analysis using the simply linked column system proved to be impractical because the gas groups (O₂ + N₂ + Ar/CO₂, N₂ /O₂/Ar) showed significantly different retention times. It is natural to consider that a difference in the column lengths may cause a pressure imbalance between the insides of the two columns resulting in the different retention times.

Therefore, Uniport B was connected to maintain a balance between the retention times. The gas mixture was analyzed on the system and a typical gas chromatograph of the gas mixture is shown in Fig. 4b. Five different peaks were successfully observed. Each peak was assigned from its retention time as A (a mixture of N₂, O₂ and Ar), B (CO₂), C (N₂), D (O₂) or E (Ar). The optimum length of

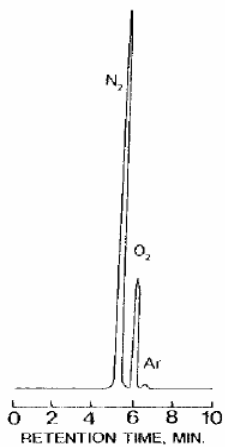


Fig.3 A gas chromatographical profile of a laboratory atmosphere on a Gaskuropack 54 column. The atmosphere was analyzed on a single Gaskuropack column (3mm×6m) at -80°C.

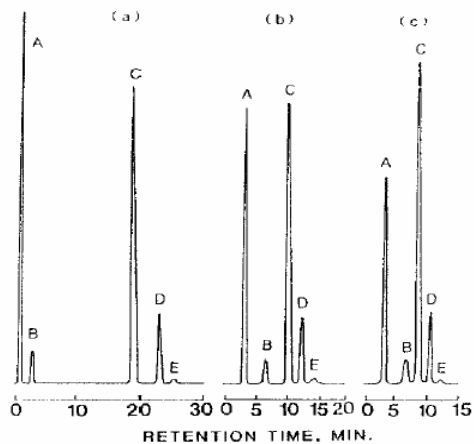


Fig.4 Effect of column length of uniport B on the resolution and retention time of inorganic gas peaks. Column lengths of Uniport B: (a) 1m, (b) 4m, and (c) 5m. Symbols are the same as those in Fig.2

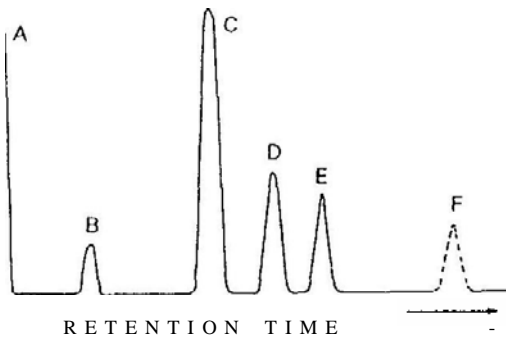


Fig.2 A schematic chromatogram expressing the complete profile of the inorganic gases including O₂, N₂, CO₂ and Ar.
 A: O₂+N₂+Ar, B: CO₂, C: N₂, D: CO₂, E: Ar, F: CO₂

Peak A and B correspond to a mixture of three gases (N₂, O₂ and Ar) and CO₂ respectively, because activated charcoal can not absorb N₂, O₂ and Ar at all. Each Peak from C to F corresponds to N₂, O₂, Ar and CO₂, respectively. Peak F is provided as a dummy for convenience of calculation because Gaskuro-pack 54 strongly absorbs CO₂ and thus no peak can be obtained from CO₂.

If it is assumed that the thermal detector shows the same response to every gas, the gas composition (%) can be expressed as follows ;

$$\text{Nitrogen (\%)} = \frac{C}{C + D + E + F} \times 100 \dots (1)$$

$$\text{Oxygen (\%)} = \frac{D}{C + D + E + F} \times 100 \dots (2)$$

$$\text{Argon (\%)} = \frac{E}{C + D + E + F} \times 100 \dots (3)$$

$$\text{Carbon dioxide (\%)} = \frac{F}{C + D + E + F} \times 100 \dots (4)$$

where C, D, E and F are the areas of Peaks C, D, E and F, respectively.

Because both Peak B/A and peak F/(C +D+E) express the ratio of CO₂ to a mixture of N₂, O₂ and Ar, the area under Peak F is calculated from equation (5),

$$F = \frac{B}{A} (C + D + E) \dots \dots \dots (5)$$

where A and B are areas of Peaks A and B, respectively. By substituting equation (5) into equations (1) to (4), the gas compositions given by the volume percentage (%) were obtained. It is not necessary to consider that the split ratio of the gas flow at the diverging point varies because the gas composition is always calculated from the ratio of each peak area (C~F) to the total peak area (C + D + E + F).

5. Relative response of TCD

The relative response of TCD to an inorganic gas was independently calibrated by determining the peak area as follows :

An accurate amount (0.5ml) of authentic gases (O₂ 99.99% ;N₂, CO₂, Ar 99.999%) was injected in the column system and total peak areas were calculated by the integrator. The averaged value of five measurements was obtained. After (C +D+E)/A (D +E~0) was calculated from the analysis of the authentic N₂, the peak area of CO₂ corresponding to Peak F was calculated by equation (5), and then B+F was considered as the total area of CO₂.

Uniport B for separating the gases on the column system was 4m (Fig. 4b). When the column was shorter than 4m, the retention time for N₂, O₂ and Ar increased significantly (Fig. 4a). On the other hand, if the column was longer than 4m, CO₂ and the gas mixture tended to overlap N₂, O₂ and Ar (Fig. 4c).

The advantage of this method is that it may allow one to inject an approximate amount of a gas mixture for determining the gas composition, and a calibration curve using authentic gases is not required.

2. Gas composition

Several authors¹⁵⁾ calculated the gas composition from areas or heights of the peaks using calibration curves of the standard gases. This procedure requires careful injection of an accurate amount of gas sample, and none of them mentions the presence of Ar in the air. While the partial pressure of each inorganic gas in a MAP system fluctuates drastically, especially during the early stage of storage, the integrated gaseous pressure inside the package is stable and is equal to the air pressure outside the package because

plastic films commonly used for this system are flexible. Therefore, the gas composition inside the package is easily obtained by measuring the ratio of each peak area (C, D, E and F) to the total peak area (C+D+E +F). Table 2 shows the gas composition of the outside atmospheric air calculated from the ratio of peak area of the gas chromatogram. The calculation procedure already mentioned did not give a reasonable result for the composition when compared with widely recognized values¹⁶⁾. Therefore, the relative responses of TCD to the gases on helium were determined. Table 3 shows the clear differences for the relative responses with small standard deviations among the four gases. A similar result has been reported by Messner et al,¹⁷⁾ covering a range of 30°C to 160°C of TCD. When the observed peak areas were divided by the relative response values, and then the gas composition of the air was calculated using equations (1) to (5), the results proved to be highly acceptable (Table 2), indicating that the discrepancy between the accepted values and the experimental values obtained this time was mainly

Table 2 The atmospheric gas composition (%) analyzed by gas chromatography.

Gas	Correction by TCD sensitivity to each gas		Values widely
	corrected	not corrected	accepted*
Nitrogen	78.08	78.95	78.03
Oxygen	20.90	19.93	20.99
Carbon dioxide	0.05	0.06	0.03
Argon	0.98	1.07	0.94

* Humphrey, 1926¹⁶⁾

Table 3 Relative responses of TCD to inorganic gases on helium.

Gas	Relative response'	
	Average	Standard deviation
Nitrogen	100	
Oxygen	94.3	0.57
Carbon dioxide	115.9	1.85
Argon	107.0	0.56

*Average of five measurements

due to the gas-dependent response of the TCD.

3. Gas composition within MAP

Changes in the gas composition in MAP of broccoli are given in Fig. 5. The packaging conditions A and C in Table 1 apply to extreme cases, because these conditions were determined to intensify the changes in either

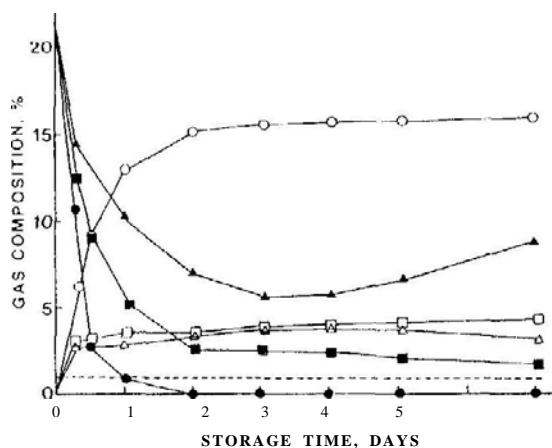


Fig. 5 Changes in O₂, CO₂ and Ar in film packages during the storage of broccoli at 15°C. The packaging conditions are given in Table 1.

Condition A (PMP) : ▲ O₂, △ CO₂, Ar
 Condition B (LDPE) : ■ O₂, □ CO₂, Ar
 Condition C (COPP) : ● O₂, ○ CO₂, Ar

O₂ or Ar concentration during storage.

When compared with other conditions, the O₂ concentration under Condition A was kept at a higher level even after 7 days. Under Condition B, O₂ gradually decreased to become equilibrated at ca. 2% after 48 hour storage at 15°C. In both conditions, color, which is important as the quality determinants of the vegetable, decreased as a function of storage time. Under Condition C, the O₂ concentration reached after 48 hour storage, and thereafter the respiration proceeded anaerobically, resulting in the generation of undesirable off-odors. But in any condition, Ar was kept in the range of 0.9 to 1.0%. If Ar had overlapped O₂ on the chromatograms, the observed concentrations of O₂, even under Condition C, would have showed almost 1 % O₂ during storage.

Some researchers have suggested that 2~5% O₂ and 6~10% CO₂ are recommended for the storage of broccoli under CA/MA¹⁸⁾¹⁹⁾. Undesirable off- odors were formed from broccoli when the produce was stored under the CA with very low O₂ (0.25% or less) at 5°C²⁰⁾. The off-odors were also developed under 1% or less O₂ in MAP²¹⁾. However, in some cases, the off-odors were also developed under relatively high concentrations of O₂ (< 0.5-4 %) ²¹⁾²²⁾.

It seems that these discrepancies in dividing the concentration between aerobic and anaerobic respirations are due in part to no separation of Ar from O₂.

Table 4 shows the gas composition in MAP of several vegetables purchased from a local supermarket. The O₂ concentrations were in

Table 4 Modified atmospheres observed in film packages for fresh produce purchased from a local supermarket.

Vegetable	Film type	Gas composition (%)			
		N ₂	O ₂	CO ₂	Ar
Shiitake fungus	PVC*	85.5	8.8	4.7	0.91
Shimeji fungus	PVC	90.6	6.1	2.2	1.10
Mung bean sprouts	LDPE**	78.7	6.0	14.4	0.89
A mix of vegetables	LDPE	79.5	5.3	14.3	0.95
Tomatoes	PVC	81.5	16.6	0.9	0.93
Cucumbers	PVC	84.6	12.7	1.8	1.01

* Polyvinyl chloride

** Low density polyethylene

*** A mix of mung bean sprouts, cabbage, carrot and black fungus.

the range of 5~16%, while the Ar concentrations were in the range of 0.9 to 1.0% again. The proper concentrations of O₂ and CO₂ in MAP were 1~2% and 10~14% for shiitake fungus at 1°C²³⁾ and 3-6% for tomatoes at 10°C²⁴⁾, respectively. The O₂ concentrations for keeping the quality of cucumbers were 3-5% at 8°C¹⁹⁾. Many other vegetables showed similar values of O₂ levels for proper packaging. Generally, the rate of respiration sensitively changes below about 8% of O₂ and above about 1% of CO₂, while a low O₂ enriched with CO₂ beyond the concentration of tolerance, induced anaerobic respiration and/or CO₂ injury²⁾.

Therefore, the respiration of the vegetables shown in Table 4 was very lightly suppressed but anaerobic respiration was not yet proceeded at all. It can be said that if the O₂ concentration had been lower, a longer shelf-life for the vegetables would have been obtained. However, if the packaging design of MAP is not based on an accurate evalua-

tion of O₂ concentration, a slight change in the storage condition may cause anaerobic respiration because suitable O₂ concentrations for the vegetables are 1~6% as previously mentioned.

Determination of O₂ concentration in MAP has used the packed column with a molecular sieve^{9)~11)22)}. However, the residual concentration of O₂ may have been overestimated because Ar always overlaps O₂ on the gas chromatogram in their systems. Therefore, the individual analysis of O₂ and Ar using the system presented herein are important. Alternatively, because it is noticed that in many cases Ar concentration is kept in the level of 0.9~1.0% as shown in Table 4 and Fig. 4, one percent O₂ should be subtracted from the values obtained by the conventional column systems.

In conclusion, the gas chromatographic procedure developed in this paper can be applied to separate the atmospheric inorganic gases from each other by a single injection and

determine them accurately. It can be expected that this technique is applicable to design for other packaging systems such as gas replacement packaging and O₂ absorber-packaging as a tool for the optimization of these packaging systems.

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