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# Effect of Modified Atmospheres on Selected Postharvest Pathogens

M.S. Mokbel and F. Hashinaga
Department of Biochemical Science and Technology, Faculty of Agriculture,
Kagoshima University, 1-21-24, Korimoto, Kagoshima, 890-0065 Japan

**Abstract:** Carbon dioxide ($CO_2$) enriched atmospheres were significantly effective to inhibit fungi growth *in vitro* and to extend the shelf life of banana fruits during storage at 20°C. Thirteen postharvest fungi were treated daily with each of the following: (a), 2, 5, 10, 20, 40, 60 kPa $CO_2$; (b) continuously with 10, 20, 40, 60 kPa $CO_2$ at 20 kPa oxygen ($O_2$) and (c) exposed only to 1 and 0.1 kPa $O_2$. The fungi were classified into high, medium and low sensitivity groups based on their growth response to $CO_2$, applied either by daily or continuously flushing. Low concentrations of $CO_2$ (2-20 kPa) showed no significant inhibition of fungal growth, while high $CO_2$ (40-60%) significantly suppressed the mycelial growth of almost all fungi of the high, medium and low sensitivity groups.

**Key words:** Carbon dioxide, postharvest pathogens

## INTRODUCTION

High levels of $CO_2$ suppresses the development of most pathogens by inhibiting various metabolic functions[1] and simultaneously delayes the ripening of fruits or vegetables[2]. Also $CO_2$ enriched atmospheres reduce the incidence and severity of decay and extend the postharvest life of strawberries[3]. Exposure of microorganisms to high $O_2$ (80 or 90% balanced with $N_2$) alone did not inhibit the microbial growth strongly, while strong inhibition was observed only when the two gases were used in combination $CO_2$ and $O_2$[4].

Controlled atmosphere storage with of above 2.8% $CO_2$ concentrations, reduced the development of lesions incited by *Botrytis cinerea* (gray mould) and *Penicillium expansum* (blue mould) in apple kept for 61 days at 0°C[5]. Super- atmospheric $O_2$ concentrations inhibited the growth of some bacteria and fungi and they were much more effective if combined with elevated $CO_2$, which is a fungistatic gas[6,7]. The commercially-used $CO_2$ level of 15 kPa in air in combination with 40 kPa $O_2$ was most effective in suppressing mycelial growth *in vitro*[7].

This study reports the effect of the application of elevated $CO_2$ and low $O_2$ at different concentrations as a possible means to control the postharvest pathogens *in vitro*.

## MATERIALS AND METHODS

**Fungi culture:** Thirteen fungi tested in this study included *Alternararia stolonifer* (Fr.) Keissl., *Penicillim expansum* Link, *Rhizopus stolonifer* (Ehrenb.) Vuill., *Geotrichum candidum* Link., *Colletotrichum acutatum* Simmonds, *Botrytis cinerea* Persoon: Fr., *Fusarium oxysporum* Schlechtend1:Fr. f. sp. *Fragariae* Winks and Williams, *Fusarium oxysporum* Schlechtend1:Fr. f. sp. *Lycopersici* (Sacc.) W.C. Snyder and H.N. Hansen, *Glomerella cingulata* (Stoneman) Spauld. and H. Schrenk, *Phytophthora citrophthora* (R.E. Sm. and E.H. Sm.) Leonian, *Monilinia fructicola* (G. Winter) Honey, *Penicillim italicum* Wehmer and *Aspergillus niger* Tiegh.

Inoculums were prepared by transferring spore from slant tube of two-weeks old colonies onto freshly prepared PDA medium plates. Hyphal tips were harvested from the edge of 6-8 days old culture (6 mm diameter and 3 mm deep) by using sterile cork borer as described by Qadir and Hashinaga[8]. The colony diameters were recorded by a digital caliper. After inoculation, the Petri dishes were placed in the jars and exposed to 10, 20, 40 and 60 kPa $CO_2$. The gases inside the jar were stirred every day by drawing and flushing back by using 60 mL gas tight syringe. The mycelial growth rate was measured after 6 days of incubation.

**Measurement of gas concentrations:** Head space concentrations of gas into the jars where measured daily for $CO_2$ and $O_2$ analyses by withdrawing 1 mL of the headspace gas from each replication by a 1 mL syringe needle. Carbon dioxide was measured by a gas chromatograph (Shimadzu, GC-8AIT) equipped with thermal conductivity detector and a 3 mm x 6 m column packed with silica gel (60/80) for $CO_2$ using helium and

**Corresponding Author:** M.S. Mokbel, Department of Biochemical Science and Technology, Faculty of Agriculture, Kagoshima University, 1-21-24, Korimoto, Kagoshima, 890-0065 Japan
Tel/Fax: +81 99 285 8666 E-mail: mo155@hotmail.com

with molecular seive 5A (3 mm x 3 m) for $O_2$ as a carrier gas (30 mL $min^{-1}$). The temperatures of the oven and injector/detector were 130 and 150°C for $CO_2$, and 50 and 80°C for $O_2$, respectively.

**Statistical analysis:** Analysis of variance was performed on the results. Data were analyzed as averages±S.D. or by two-way analysis of variance (ANOVA) with mean separation by Fisher PLSD test ($p \leq 0.05$).

## RESULTS

**Fungi culture:** Percentage inhibition of colony generally increased significantly (p=0.05) with increasing $CO_2$ concentrations in all groups of fungi. Fungal growth response to $CO_2$ would be divided into high, medium and low sensitive groups (Table 1). Carbon dioxide at 2 and 5 kPa in all groups did not significantly (p=0.05) reduce the growth of fungi *in vitro*. The growth rate of the high sensitivity group was inhibited with increasing $CO_2$ and completely inhibited at more than 40 kPa $CO_2$. The growth reduction of fungi in the medium sensitivity group ranged between 70 to 79% at 40 kPa and almost completely inhibited at 60 kPa. On the other hand, the growth reduction of fungi in the low sensitivity group ranged between 63 to 67% only at 40 kPa and the growth reduction ranged between 78-86%. At high concentration of $CO_2$, the fungi growth of the high sensitivity group was completely inhibited by 60 kPa $CO_2$, while only 86% of fungi growth of the low sensitivity group was inhibited.

Continuous exposure of $CO_2$ to fungi *in vitro* resulted in more effective inhibition than that of intermittent daily flushing (Table 2). Moderate inhibition of most fungi growth was observed at low gas concentrations of 10-20 kPa $CO_2$. Increasing the $CO_2$ concentrations (40-60 kPa) inhibited most fungi growth rates.

Table 1: Percent inhibition of colony growth of fungi flashed daily with $CO_2$ for 6 days at 20°C

| | $CO_2$ concentrations (%) | | | | | |
|---|---|---|---|---|---|---|
| Fungi | 2 | 5 | 10 | 20 | 40 | 60 |
| **High sensitive group** | | | | | | |
| *Aspergillus niger* | 3±0.3c | 14±0.3b | 26±0.3b | 74±0.3a | 100±0.0a | 100±0.0a |
| *Fusarium oxysporum* f. sp. *lycopersici* | 5±0.3b | 10±0.3c | 19±0.3c | 71±0.3b | 100±0.0a | 100±0.0a |
| *Monilinia fructicola* | 6±0.0a | 17±0.3a | 27±0.3a | 70±0.3c | 92±0.7b | 100±0.0a |
| *Botrytis cinerea* | 0±0.0d | 0±0.0h | 8±0.0j | 67±0.3d | 96±0.0a | 100±0.0a |
| **Medium sensitive group** | | | | | | |
| *Rhizopus stolonifer* | 0±0.0d | 0±0.0h | 0±0.0l | 30±0.3j | 70±0.7de | 96±2.8bc |
| *Geotrichum candidum* | 0±0.0d | 4±0.0e | 14±0.3g | 42±0.3e | 79±0.0c | 93±0.0c |
| *Colletotrichum acutatum* | 0±0.0d | 6±0.0d | 15±0.3f | 33±0.3h | 73±0.7dc | 90±0.0c |
| *Alternaria alternata* | 0±0.0d | 3±0.3f | 18±0.3d | 37±0.3g | 76±0.7c | 90±2.1c |
| *Penicillium expansum* | 0±0.0d | 0±0.0h | 13±0.0h | 39±0.3f | 76±0.7c | 93±0.7c |
| *Glomerella cingulata* | 0±0.0d | 6±0.0d | 16±0.3e | 31±0.3i | 77±0.7c | 92±2.8c |
| **Low sensitive group** | | | | | | |
| *Penicillium italicum* | 0±0.0d | 2±0.7g | 9±0.3i | 26±0.3l | 63±0.7e | 86±2.8d |
| *Phytophthora citropthora* | 0±0.0d | 2±0.0g | 7±0.0k | 21±0.3m | 67±1.0e | 86±0.0d |
| *Fusarium oxysporum* f. sp. *fragariae* | 0±0.0d | 2±0.0g | 7±0.0k | 27±0.3k | 64±0.8e | 78±2.8e |

The fungi were grown in PDA medium and the colony growth (mm/day) of three plates for each replication each were measured twice. The radius in each plate was measured toward three different directions from the center of the colony. Control fungi were grown in the air. Means separation within each columns followed by different letters are significantly different as determine by Fisher PLSD test (p=0.05) Values shown are means±S.D of 9 plates for each three replication each were used for every measurement

Table 2: Effects of $CO_2$ and $O_2$ and on fungi growth (inhibition %)

| | $CO_2$ kPa | | | | $O_2$ kPa | | $N_2O$ kPa (x) | | |
|---|---|---|---|---|---|---|---|---|---|
| Fungi | 10 | 20 | 40 | 60 | 1 | 0.1 | 30 | 50 | 80 |
| *Aspergillus niger** | 33.3±0.7a | 66.7±0.3b | 100.0±0.0a | 100.0±0.0a | 8.3±0.0e | 16.7±0.3e | * | * | * |
| *Monilinia fructicola* | 27.0±0.7b | 51.4±0.3d | 91.9±0.3c | 100.0±0.0a | 18.9±0.3c | 29.7±0.0c | 71 | 100 | 100 |
| *Botrytis cinerea* | 16.9±0.7d | 63.4±0.7c | 94.4±0.3b | 100.0±0.0a | 4.2±0.0f | 23.9±0.0d | 96 | 98 | 100 |
| *Penicillium exponsum* | 11.8±0.0e | 35.3±0.7f | 70.6±0.4g | 94.1±0.3d | 0.0±0.0g | 0.0±0.0f | 65 | 87 | 100 |
| *Penicillium italicum* | 9.1±0.7g | 27.3±0.3i | 63.6±0.4h | 88.6±0.3e | 0.0±0.0g | 0.0±0.0f | 87 | 93 | 100 |
| *Colletotrichum acutatum* | 15.8±0.7d | 36.8±0.3e | 73.7±0.3e | 91.6±0.7de | 0.0±0.0g | 0.0±0.0f | 40 | 60 | 100 |
| *Rhizopus stolonifer* | 0.0±0.0j | 22.4±0.3j | 75.5±0.3d | 91.8±0.3de | 0.0±0.0g | 0.0±0.0f | 100 | 100 | 100 |
| *Phytophthora citrophora* | 4.0±0.0h | 20.0±0.0k | 60.0±0.0i | 88.0±0.3e | 24.0±0.7b | 36.0±0.3b | 45 | 76 | 100 |
| *Fusarium oxysporum* f. sp. *lycopersic* | 22.2±1.4c | 77.8±0.7a | 100.0±0.0a | 100.0±0.0a | 11.1±0.0d | 29.7±0.3c | 24 | 30 | 57 |
| *Geotrichum candidum* | 11.1±0.7f | 37.0±0.3e | 74.1±0.3e | 92.6±0.7d | 29.6±0.3a | 40.7±0.3a | 4 | 15 | 55 |
| *Alternaria alternata* | 12.5±1.4e | 33.3±0.7g | 70.8±0.3g | 95.8±0.4b | 0.0±0.0g | 0.0±0.0f | 9 | 32 | 57 |
| *Glomerella cingulata* | 12.0±0.0ef | 32.0±0.3g | 72.0±0.0f | 96.0±0.3b | 0.0±0.0g | 0.0±0.0f | 41 | 59 | 73 |
| *Fusarium oxysporum* f. sp. *fragariae* | 8.6±0.7g | 28.6±0.3h | 62.9±0.7j | 85.7±0.7e | 0.0±0.0g | 0.0±0.0f | 24 | 30 | 57 |

The fungi were grown in PDA medium and the colony growth (mm/day) of three plates for each replication each were measured twice. The radius in each plate was measured toward three different directions from the center of the colony. Control fungi were grown in the air. Means separation within each columns followed by different letters are significantly different as determine by Fisher PLSD test (p <0.05). Values shown are means±S.D of 9 plates for each three replication each were used for every measurement. (x) Source: Qadir and Hashinaga[8]. **Aspergillus niger* was out of measurement under $N_2O$

## DISCUSSION

**Fungi culture:** These studies demonstrate the ability of high $CO_2$ levels to inhibit effectively fungal growth when $O_2$ level was 20%. The fungi growth was inhibited with a high concentration of $CO_2$. The growth rate of fungi increased immediately upon removal from the controlled atmosphere, indicating that there was no residual inhibitory effect of $CO_2$ treatments. This variation in inhibition of fungi growth suggests that $CO_2$ may have suppressed the fungal metabolic activity in cell wall tissue. Tian *et al.*[9] showed that the growth of *M. fructicola* significantly declined with increasing $CO_2$ concentrations. Paterson and Jones[10] observed that at 50% $CO_2$, the development of brown rot caused by the growth of *M. fructicola* on all fruits was completely inhibited for a period of 7 days at 20°C.

In fact present results indicated that the growth of *M. fructicola* was inhibited 92% by flushing daily with 40% $CO_2$ for a period of 6 days at 20°C. Paster and Bullerman[11] reported that low $O_2$ concentrations (less than 1%) and increased concentrations of $CO_2$ or $N_2$ were found to be highly effective in preventing the development of mould on grain and in inhibiting the selected mycotoxins. Low $O_2$ concentrations (1 and 0.1 kPa) *in vitro* were not significantly effective in fungi growth rate in all groups. However, the effectiveness of $CO_2$ in inhibition the mycelial growth of fungi was more than that of $N_2O$. These results correspond with the data of Sitton and Patterson[5]. Actually, the fungi grew significantly under low $O_2$ concentration. Wszelaki and Mitcham[7] proposed that elevated $O_2$ affects more the growth of *B. cinerea*, and is more significant at 100 kPa $O_2$ level which inhibited mycelia growth. The effectiveness of $CO_2$ on inhibition of fungi growth was similar to that of $N_2O$ on biophysical properties, which maybe due to uptake of $N_2O$ and $CO_2$ into cell tissue of fungi. This is in agreement with studies by Qadir and Hashinaga[12], Gouble *et al.*[13]. The concentration of $CO_2$ required inhibiting mycelial growth varied with fungal species. The growth of mycelium of fungi decreased linearly with increasing $CO_2$ concentration from 5 to 60%.

## CONCLUSION

These results effectively demonstrate the inability of fungi to grow at high concentrations of $CO_2$ when $O_2$ level is 20%. Most of mycelia grew slowly with elevated $CO_2$. The growth rate increased immediately upon removal from the treating atmosphere, indicating that there was no residual inhibitory effect of $CO_2$ treatments.

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