MODIFIED ATMOSPHERES TO DELAY SENESCENCE AND DECAY OF BROCCOLI

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Introduction

Storability of the broccoli flower heads is very low, especially at temperatures above 5°C. Respiration rates increase sharply and the inflorescence turn yellow due to opening of the flower buds and degradation of chlorophyll in the green tissues. This may be followed by drop of the flowers. In an advanced stage of senescence, decay caused mainly by Alternaria alternata and Botrytis cinerea, starts to develop (3).

During the last 45 years many studies have shown the senescence-retarding effect of high CO_2 and low O_2 in the atmosphere of broccoli flower heads (5-9,12,13,15,16). Oxygen concentrations under 1% are needed to retard completely senescence of broccoli flower heads whereas a wide range of CO_2 concentrations between 5-20% were effective. Increasing CO_2 concentrations was much more effective than decreasing O_2 concentrations. In spite of the beneficial effect of CO_2 its mode of action in retarding senescence of the broccoli flower heads is still obscure. So far, modified atmospheres have not been used commercially for broccoli, probably, because of the possibility of offensive odor and off-flavors which might occur under insufficient aeration (5,6,8,9,13,15,16).

Inspite of the high respiratory activity we have found that ethylene production rates of the entire flower head are relatively low. However, the flower buds are most vulnerable to the adverse effects of ethylene and a few ppm of the gas in the atmosphere enhance yellowing, bud drop and decay. Lieberman and Hardenburg (7) have suggested a possible role for ethylene in the yellowing of broccoli heads. Deterioration of broccoli was retarded by ethylene inhibitors (14). A primary role for ethylene in the senescence of leaf tissues has been demonstrated later on by Aharoni and Lieberman (1).

The purpose of our investigation was to ascertain the regulatory role of ethylene in the senescence of broccoli inflorescence and to

examine the possibility of using modified atmosphere to retard senescence and decay of broccoli flower heads in long distance transit.

Procedures

Experiments were carried out with either flower buds or entire heads of broccoli (Brassica oleracea L. var. Italica cv. Green Duke). In laboratory tests, in which the regulatory role of ethylene was studied, flower buds with stalk portions, weighing about 1 gram, were surface-sterilized with 0.1% NaOCl and 0.2% Rovral. All subsequent handling of the tissues involved sterile techniques. The flower buds were enclosed in 50-ml Erlenmeyer flasks containing 0.5 ml H_2O. Two plastic center well were hung in each flask. One contained a filter paper wick wetted with 0.1 ml of 0.25 M $Hg(ClO_4)_2$ reagent for ethylene absorption, and the second one contained a wick wetted with 0.1 ml of 10% KOH for CO_2 absorption. The flasks were sealed with rubber serum caps and incubated in darkness at 25° or 30°C. CO_2 evolution, ethylene production and ACC content were assayed periodically. Where indicated the $Hg(ClO_4)_2$ or KOH solution were omitted and pure ethylene or CO_2 were injected into the sealed flasks. The ethylene absorbed to the $Hg(ClO_4)_2$ solution, and the CO_2 absorbed to the KOH solution were measured as previously described (2). ACC was extracted as described by Riov and Yang (11) and the amount in each extract was assayed by the methods of Lizada and Yang (10).

Role of Ethylene in Senescence of the Broccoli Inflorescence

The rate of ethylene production by the entire broccoli head was very low. During the course of senescence, a rise in ethylene production, in a climacteric-like pattern, was observed regardless of the size of the tissue cut from the head (Fig. 1). However, the smallest inflorescence portions, weighing about 1 gram, produced much more ethylene per gram fresh weight. This indicates that the flower buds, rather than the surrounding vegetative tissues, are responsible for most of the ethylene production measured.

We studied the regulatory role of endogenous ethylene in the senescence of broccoli florets by applying exogenous ethylene at a concentration of 10 ppm. After 130 h of incubation the level of chlorophyll was very low in the untreated tissue and exogenous ethylene slightly enhances its degradation (Table 1). Treatment of the florets with AVG, an inhibitor of ethylene biosynthesis, remarkedly reduced chlorophyll destruction. CO_2, Ag^+, benzyl adenine and gibberellic acid, which are known to block ethylene action in the

Table 1. Effect of ethylene on the senescence of broccoli floretts.

Treatment*	$-C_2H_4$	$+C_2H_4$(10µl/l)
	Chlorophyll (% of initial**)	
Control	35	29
AVG(0.1mM)	75	28
CO_2(10%)	60	38
$AgNO_3$(10µl/l)	58	22
BA(0.5 µm)	62	20
GA_3 (0.5 µm)	61	32

*AVG, BA and GA_3 were added to the incubation medium; $AgNO_3$ was applied by pretreatment for 45 min.
**Chlorophyll was extracted with 80% ETOH after 130h of incubation.

senescence process, were also very effective. Since the effect of all these agents in retention of chlorophyll was completely abolished by 10 ppm of ethylene it is concluded that ethylene plays a primary role in the initiation of the senescence of the broccoli inflorescence, presumably, by interacting with other hormones participating in the process.

Effect of CO_2 on Respiration and Ethylene Biosynthesis

CO_2, being a naturally occurring compound, and most effective in blocking ethylene action in the senescence of broccoli florettes, was further studied in order to elucidate its mode of action in the retention of chlorophyll. The results given in Figure 2 indicate that the inhibitory effect of CO_2 in the degradations of chlorophyll cannot be ascribed to suppresive effect on respiration. On the contrary, CO_2 unexpectedly, even increased respiration rates during the first 3 days of incubation. We assume that this could be related to the recently reported effect of CO_2 in stimulating ethylene biosynthesis in leaf tissue (1,4) which in turn could cause an increased repiration rates. This assumption is supported by data shown in Table 2. As was found with leaf tissue, CO_2 stimulated ethylene production in broccoli floretts (Fig. 3A). This stimulation was most pronounced during the first 3 days of incubation. The stimulatory effect of CO_2 stems from enhancement of both ACC formation (Fig. 3B) and the conversion of ACC to ethylene (Fig. 4).

Table 2. Effect of ethylene on respiration rates of the broccoli floretts.

Incubation time (h)	CO_2 evolution(µl/g/h)	
	$-C_2H_4$	$+C_2H_4$(10µl/l)
20	448	485
40	467	524
60	470	490
80	531	581
100	354	407

CO_2 Retards Development of Decay

The effect of CO_2 and ethylene on rot development following inoculation of the florettes with a spore suspension of Botrytis cinerea is shown in Table 3.

Table 3. Effect of CO_2 and ethylene on the decay developed on broccoli floretts inoculated with Botrytis cinerea spore suspension.

Treatment	Index of Decay*
Control	4.0
CO_2(10%)	2.6
C_2H_4(10µl/l)	4.2
CO_2 + C_2H_4	4.2

*Decay was evaluated after 120 h of incubation. 1 = non; 5 = maximum development.

A marked decrease in rot development was observed after floretts held in CO_2-enriched atmosphere. This inhibitory effect was completely nullified by addition of 10 ppm ethylene to the CO_2-treated florettes. In other experiments we have found that 12% CO_2 caused a decrease of 25 and 15% in mycelial growth (in vitro) of Botrytis

cinerea and Alternaria Alternata, respectively (3). Since less than 10% CO_2 was sufficient for complete inhibition of fungal development on the broccoli we conclude that the suppression of rot development is related mainly to the effect of CO_2 in retarding the senescence processes in the host tissue, thereby, maintaining its natural resistance to decay.

Respiratory CO_2 Retards Yellowing and Decay in Stored Broccoli Packed in Sealed Bags

Since the respiration rate of the harvested broccoli flower heads is extremely high we have studied the effects of the modified atmospheres inside sealed polyethylene bags (0.04mm) containing broccoli flower heads during simulated prolonged transit (Fig. 5). Respiratory CO_2 increased in the sealed bags up to 5% during the first 48 h in a cold room and thereafter, gradually decreased to about 2.0%. After transfer of the broccoli to 20°C, the CO_2 concentration increased to 6%. The oxygen concentration dropped to 10% during the first 24h, but gradually increased to 16% during 24 days in cold storage. Holding the stored broccoli for 2 days at 20°C caused a decreased in O_2 concentration to about 6%.

The quality of the packed broccoli heads after simulated transit by either air or sea surface is shown in Table 4.

Table 4. Quality of broccoli heads packed in either PVC wraps or PE-sealed bags.

Simulated Transit	Treatment	Yellowing Index*	% decay
Air 3 days at 15°C +2 days at 20°C	PVC Wrap	4.8	8.5
	PE-sealed bag	1.0	0
Sea Surface 14 days at 0.5°C +2 days at 20°C	PVC Wrap	4.1	32.0
	PE-sealed bag	1.1	0

*1 = non; 5 = maximum yellowing.

In both cases, when the heads were exposed to severe transit conditions, the heads packed in sealed PE bags remained green and fresh whereas, the PVC-film wrapped heads were unsalable. In other experiments (not shown) we found that broccoli heads in sealed PE bags remained in excellent condition for 4 weeks at 0.5°C followed by an additional 2 days at 20°C. Neither offensive odor nor off-flavors were detected in all the experiments performed.

Conclusions

(1) It is clearly shown that endogenous ethylene plays a primary role in the regulation of senescence processes of broccoli inflorescence.

(2) The senescence-retarding effect of CO_2, gibberellic acid and benzyl adenine is related to their ability to block ethylene action.

(3) Modified atmospheres created in sealed PE bags during storage and transit allowed the broccoli heads to be kept in high quality for more than 3 weeks in cold storage followed by at least 2 additional days at 20°C.

(4) The accumulation of respiratory CO_2, rather than O_2 depletion, was found to be the main factor controlling the keeping quality of the stored broccoli.

(5) The prevention of decay was related to the senescence-retarding effect of the accumulated CO_2 rather than to a direct effect of the gas on fungal development.

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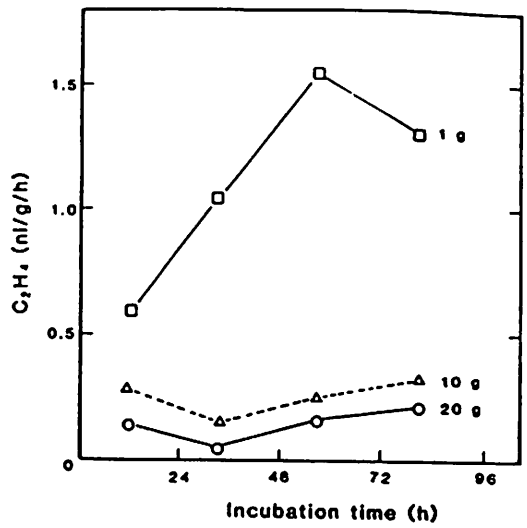

Figure 1. Effect of tissue weight on ethylene production rates.

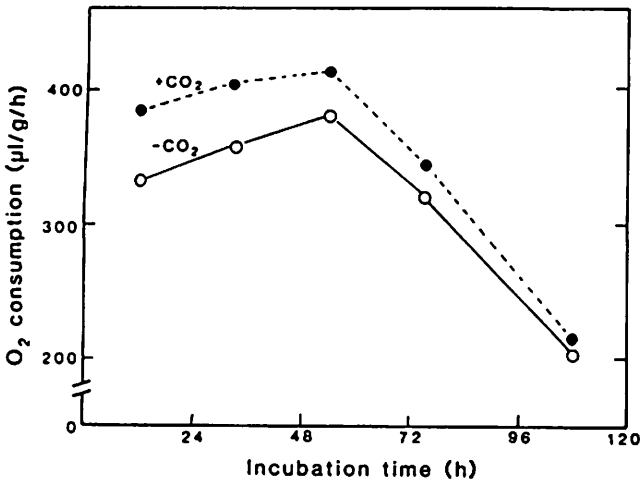

Figure 2. Effect of CO_2 (10%) on the respiration rate.

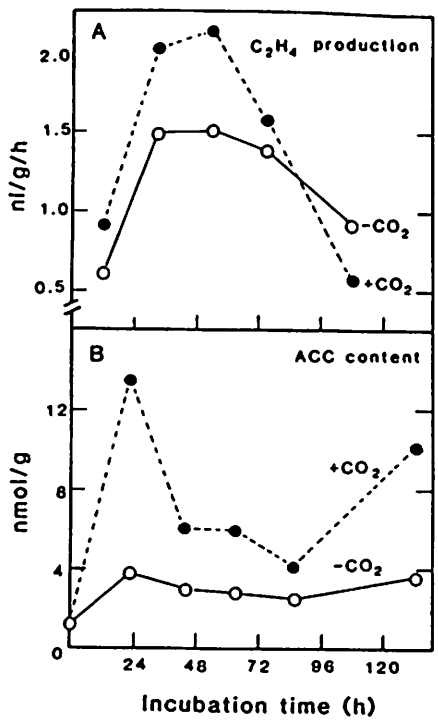

Figure 3. Effect of CO_2 (10%) on ethylene production and content of ACC