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Physiological and Quality Responses of Brussels Sprouts to Storage in Controlled Atmospheres

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Abstract. **Brussels sprouts (*Brassica oleracea* L., Group *gemmifera*) were stored 2, 3, or 4 weeks at 2.5°, 5°, or 7.5°C with 0.5%, 1%, 2%, 4%, or 21% O_2, or in the following combinations of $\%O_2/\%CO_2$: 1/10, 2/10, or 20/10. Storage was followed by 2 or 3 days of aeration at 10°. Low O_2 levels reduced the rate of CO_2 production relative to that in air, but rates were similar among the low O_2 levels. Ethylene production was low at 2.5° and 5° in all atmospheres, but at 7.5° it was 20% to 170% higher in air than in low O_2. Ethylene production was virtually stopped during exposure to high CO_2, but increased dramatically during aeration. Since low O_2 levels retarded yellowing and 10% CO_2 retarded decay development, the combinations of low O_2/high CO_2 effectively extended the storage life of the sprouts at 5° and 7.5°. The beneficial effect of CA storage was still evident after return of the samples to normal air. The sprouts retained good appearance for 4 weeks at 2.5° whether stored in CA or in air. Storage in 0.5% O_2 occasionally induced a reddish-tan discoloration of the heart leaves and frequently an extremely bitter flavor in the nongreen portion of the sprouts. None of the atmosphere modifications appreciably affected either the tissue pH or texture of the sprouts.**

Brussels sprouts store well for several weeks at 0°C in air (19), especially when the relative humidity is near 100% (27). However, such ideal conditions are virtually impossible to maintain during marketing. The influence of controlled atmosphere (CA) storage on quality retention in brussels sprouts has been investigated repeatedly (8, 20, 22–24, 28). Generally, O_2 at about 2% and/or CO_2 at 5% or higher retarded yellowing. High CO_2 also tended to retard discoloration of the cut end of the sprouts and decay development. We conducted our studies to determine: a) optimal CA conditions for brussels sprouts held at higher than ideal temperatures (about 0°); b) the response of brussels sprouts to very low O_2; c) the influence of low O_2 and/or high CO_2 concentration on ethylene evolution by the sprouts; d) the effect of various O_2/CO_2 combinations applied to raw sprouts on pH and texture when cooked; and e) whether the green and nongreen portions differ in response to CA conditions.

Materials and Methods

Source and handling of sprouts. The brussels sprouts ('Lunette', 'Rampart', or 'Valiant') were grown and packed commercially in the central areas of coastal California between September and February of several years. The packed sprouts were covered with crushed ice, transported to Fresno, and held overnight at 2.5°C. After eliminating sprouts with major defects, two samples, each consisting of at least 25 similar-sized sprouts and weighing 600 to 700 g (± 1 g) were placed in glass jars. One sample was examined after 12 or 13, 19 or 20, or 26 or 27 days of storage at 2.5°, 5°, or 7.5° (± 0.5°) under the CA conditions to be noted. The other sample was examined after having been held an additional 2 or 3 days in air at 10°. All jars were at the selected temperature within 27 hr of harvest.

Atmospheres. The influence of low $-O_2$ atmospheres was tested in one series of experiments by holding the sprouts in 0.25%, 0.5%, 1%, 2%, 4%, or 21% (air) O_2. Since injury was severe in 0.25% O_2, this atmosphere was omitted in later tests. In analogous tests, the sprouts responded similarly to 10%, 15%, and 20% CO_2 when combined with normal or low O_2 (0.5% or 1%). Therefore, we evaluated 1%, 2%, or 20% O_2 with 10% CO_2 in a second series of tests. These O_2/CO_2 combinations were tested against 1/0 and 2/0. No 20/0 control was needed, because the low-O_2 tests already had shown that deterioration was most rapid in air.

All gas mixtures were humidified to near saturation by passing them through glass tubes (≈25 × 450 mm) that held a tube of filter paper standing in 1% aqueous $Cu(SO_4)_2$ at 20° ± 2°C. The salt prevented growth of microorganisms on the paper. Condensate was collected in test tubes placed in the cold rooms before the humidified gases passed into the jars. The samples held for 4 weeks were used to monitor CO_2, and ethylene production and results are given for one test at each temperature. All further experimental details related to gas mixing, flow rate, monitoring, and analysis have been described (14, 16).

Sample evaluation. The sprouts were evaluated initially for general appearance, and 10 to 20 were examined internally. Each stored sample was weighed and individual sprouts were rated for severity of decay and condition of the cut end on a scale where 1 = none and 9 = extreme (9). External appearance (a summary rating) was influenced primarily by degree of greenness and presence of decay and, to a lesser degree, by wilting and black speck and was expressed on a scale where 9

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= excellent to 1 = inedible (9). Buds rated 5 or higher for external appearance were considered salable. Butt color was recorded only in qualitative terms and callus as present or absent.

The sprouts that were evaluated raw were trimmed of any discolored tissue and then cooked 5 min in 2.5 liters of water that contained 25 g of table salt. Timing was started when the water reached 97°C (18). After the samples had cooled to laboratory temperature, we evaluated the texture of 10 cooked sprouts of similar size by means of an Instron Universal Testing Machine equipped with a 7.9-mm-diameter cherry pitter (18).

pH was determined (Corning 125 pH meter with a flat-surface combination electrode) on sprouts used for texture measurements following 60 sec of blending and 15 sec of equilibration (5).

Special low-O_2 tests. The possibility that the sporadic occurrence of severe bitterness in sprouts stored in 0.5% O_2 might be related to the density of the sprouts was tested in five experiments. In these tests, half the sprouts for each of two jars were stored whole, while the remainder were halved down to the stem to obviate the possibility of inadequate diffusion of O_2 to the center of the sprouts. In three of the tests, sprouts placed in a plastic-covered glass dish were cooked 4 min in a microwave oven. About 20 ml of H_2O was placed in the bottom of the dish. The senior author then judged the cooked sprouts for bitterness.

Statistical treatment. Each atmosphere (five each for low O_2 and O_2/CO_2 tests) was evaluated in two tests (replications) at each of three temperatures and three storage periods. Variance stabilizing transformations were determined by the method given by Box et al. (3). Percent decayed buds was transformed by logs for the low-O_2 tests and by square roots for the O_2/CO_2 tests. Reciprocal square roots were used for the Instron texture readings for the O_2/CO_2 tests. Cochran's procedure was used to test for homogeneity of variances (1). Analyses of variance were computed by using the SAS, GLM procedure using the pooled interactions of atmospheres, temperatures, and storage periods with tests as the error term (26). The Marquardt method was used to fit the asymptotic models (21). Significance testing was at the 5% level.

Results

All cultivars used responded similarly to the various treatments and thus are not distinguished hereafter.

CO_2 production. The rate of CO_2 production 24 hr after the sprouts were placed in storage (initial rate) increased with increasing temperature (Fig. 1). The rates at all three temperatures were lower for sprouts held with low O_2 than for those held in air. Differences in rates among sprouts held with 0.5%, 1%, 2%, or 4% O_2 were relatively small. The downward respiratory drift for all atmospheres was similar at 2.5° and 5°C (Fig. 1 B and C), but the rate increased substantially after 6 days for sprouts held in air at 7.5° (Fig. 1A). (The unreasonably high rates of CO_2 production at 5 days and 2.5° are unexplained.) The rate of CO_2 production increased in all samples after their transfer to 10° in air, save that of the controls from 7.5°, in which the rate decreased.

Six hours after removal of the samples from the low O_2/high CO_2 combinations at 2.5°, 5°, or 7.5°C to air at 10°, samples that had been held with 10% CO_2 produced two to three times as much CO_2/hr as samples that had been held in low O_2 only (Fig. 2).

Ethylene production. Ethylene production followed no discernible pattern during storage of the sprouts at 2.5° or 5°C, with rates mostly remaining below 0.25 $\mu l \cdot kg^{-1} \cdot hr^{-1}$. However, after 19 days at 7.5° (Fig. 3), the rates for samples held in air were from 20% to 170% higher than for those held in 2% and 0.5% O_2, respectively. Except for the air control, the rate of ethylene production increased further after the sprouts were transferred to 10° and air.

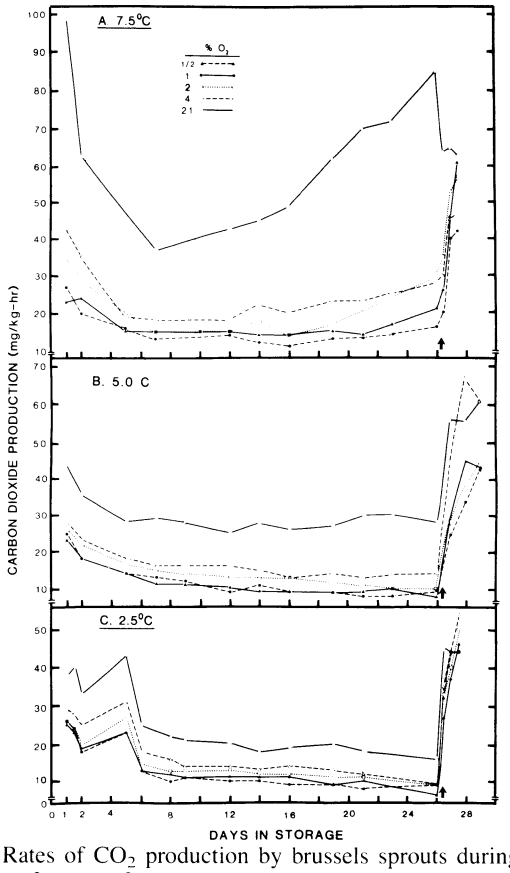

Fig. 1. Rates of CO_2 production by brussels sprouts during storage at 2.5°, 5°, or 7.5°C and in various O_2 concentrations and after transfer (arrows) to 10° in air. Rates at the three temperatures represent different tests and thus are not directly comparable. Each data point represents a single reading.

Ethylene production was at or under the limit of detectability (<0.01 $\mu l \cdot kg^{-1} \cdot hr^{-1}$) in all samples while they were stored with 10% CO_2. Removal of the samples from CA conditions at 2.5°, 5°, or 7.5°C to air and 10° was associated with a substantial increase in ethylene production in some of the samples (Fig. 2 D–F). The change in rate for all those from 2.5° (Fig. 2D) followed a similar pattern: a sharp increase right after transfer, followed by an equally sharp decrease to near or below the detectable level. Samples held at 5° with 10% CO_2 produced no detectable amount of ethylene during the first 2.5 days of aeration; on the third day, production increased decidedly only in the sample from 20/10 (Fig. 2E). In contrast, ethylene production increased sharply in samples that had been held in 1% or 2% O_2 without CO_2. The overall pattern of ethylene production in samples from 7.5° (Fig. 2F) was similar to that for samples from 5°.

Weight loss. Total weight loss generally was <2%, with 0.5% or less occurring during aeration at 10°C. The highest total weight loss was 3% in one lot at 5°C and consisted of 0.5% carbon (27% of CO_2 evolved, based on rate of 28 mg $CO_2 \cdot kg^{-1} \cdot hr^{-1}$) and 2.5% water. These percentages for 26 days are close to

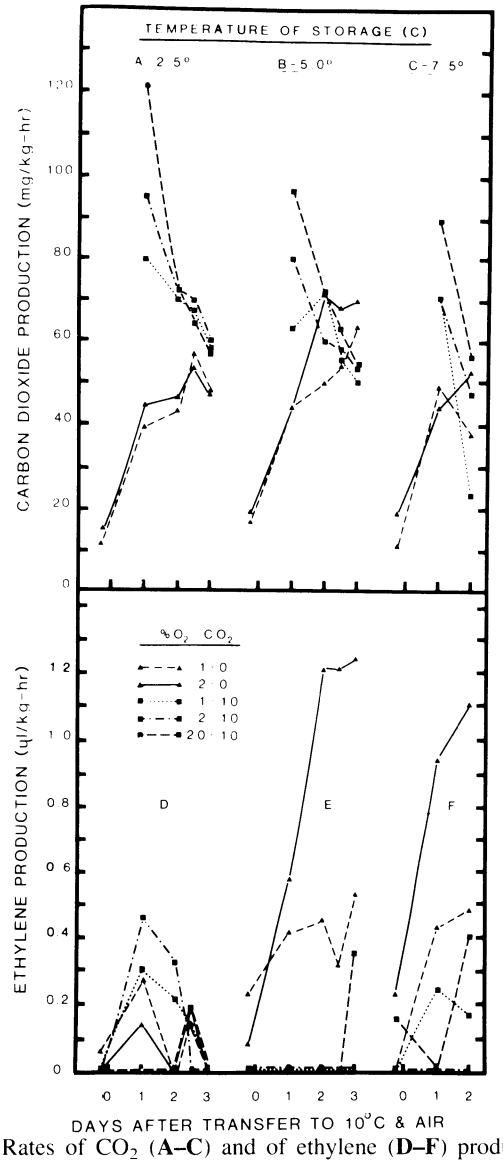

Fig. 2. Rates of CO_2 (**A–C**) and of ethylene (**D–F**) production by brussels sprouts after their transfer from low temperatures and various O_2/CO_2 concentrations to 10°C and air. Rates for the three temperatures represent different tests and thus are not directly comparable. Each data point represents a single reading.

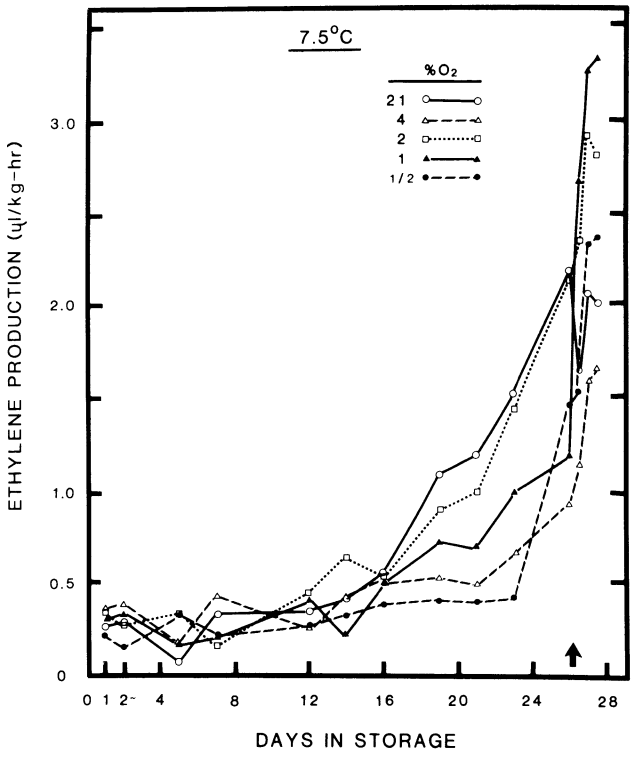

Fig. 3. Rates of ethylene production by brussels sprouts during storage at 7.5°C and in various O_2 concentrations and after transfer (arrow) to 10° in air. Each data point represents a single reading.

those calculated by Gaffney et al. (7) for 30 days at 99% RH, i.e., 2.9%, 0.7%, and 2.2%, respectively.

Quality aspects

Decay. Lowering the O_2 level to 1%, 2%, or 4% did not reduce the development of decay relative to the controls. A decay reduction due to storage in 0.5% was evident after aeration (13% in air, 4% in 0.5% O_2), but is of no practical value due to low-O_2 injury.

High CO_2 significantly reduced decay development relative to low O_2 alone. After cold storage, the incidence was <2% in 1/10 or 2/10, but between 6% and 8% for 1/0 and 2/0. The incidence for 20/10 was intermediate and significantly different only from 2/0. The incidence of decay about doubled in all lots during aeration.

The level of decay generally increased with temperature and duration of storage, but never exceeded 15% in any treatment and was virtually absent in all samples stored at 2.5°C with 10% CO_2.

Butt condition. Levels of 1% O_2 or higher were only minimally effective in retarding darkening of cut surfaces. Variation within a given treatment was high and only 0.5% O_2 consistently retarded darkening, just as in lettuce (11). Tan or gray discoloration of the cut end was retarded slightly for sprouts stored with 1/0, 2/0, or 4/0 at 2.5°C, but not for those at 5° or 7.5°. Low temperature more effectively retarded darkening than low O_2 concentration. The butts of sprouts stored at 2.5° were still mostly white to light gray after 3 weeks, whereas those of sprouts stored at 5° or 7.5° were light tan or gray after only 2 weeks. Darkening continued during the two or three days at 10° in air, so that cut surfaces finally ranged from tan/gray to brown/black.

In contrast to low O_2 levels, 10% CO_2 retarded darkening of the butts for sprouts stored at 2.5° or 5°C, but only marginally for those at 7.5°. The butts of sprouts harvested in fall and stored at 5° or 7.5° tended to be gray after storage, whereas those of sprouts harvested in winter tended to be tan or brown.

Callus grew from the parenchyma tissue centripetal to the vascular ring in some tests, sometimes to a height of 2 or 3 mm. The callus: a) grew less prolifically in 2% or at lower O_2 levels or with 10% CO_2 than in air; b) responded inconsistently to temperature; c) was present mainly after 4 weeks of storage; and d) only in sprouts harvested in fall. This seasonal effect may be analogous to environmentally induced variations in the formation of adventitious buds or roots (2).

General external appearance. Storage of brussels sprouts with 0.5%, 1%, or 2%, instead of with 21% O_2 or with 10% CO_2, substantially retarded visible deterioration. The relationship between the percentages of salable sprouts and O_2 concentration was asymptotic immediately after CA storage (Fig. 4) and after aeration (data not shown). The curve reflects the general ob-

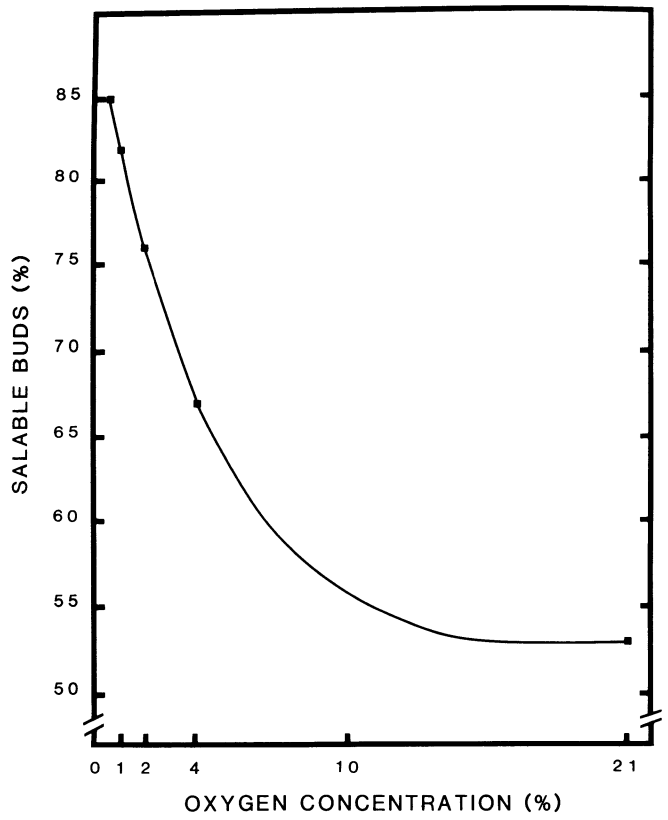

Fig. 4. Dependence of the percentage of salable brussels sprouts on O_2 concentration during storage. Asymptotic relationship: $y = k + ab^x$, where k = estimate of asymptote, k + a = intercept, and b = slope in ln scale; k = 52.6; a = 36.3, b = 0.803. Each data point represents the mean of 18 observations.

Table 1. Influence of duration and temperature of storage on the percentage of salable brussels sprouts (low-O_2 tests only).[z]

Duration of storage (weeks)	Salable buds (%) Temp (°C) 2.5	5.0	7.5
2	98	90	76
3	97	72	45
4	86	60	30
LSD 0.05 (all temperatures) = 16			
SE 0.05 = 5.5			

[z]The interactions with O_2 concentration were not significant. Each datum is the mean of 10 observations.

servation that the benefits of low O_2 levels for many crops fade above ≈4% to 5% O_2 (25).

The proportion of salable sprouts decreased with duration of storage, but much less at 2.5°C than at 5° or 7.5° (Table 1).

Carbon dioxide and storage period had a significant interactive effect on the proportion of salable buds since the influence of 10% CO_2 was evident only after 4 weeks of storage. At that time, nearly all sprouts held with 10% CO_2 were salable, but only about 70% of those held in low O_2 without CO_2 (Table 2A). Prior storage with 10% CO_2 effectively retarded deterioration during aeration, a benefit that was particularly evident among sprouts that had been stored 4 weeks (Table 2B).

pH and texture. O_2 concentration in the storage atmosphere did not influence the pH of the cooked sprouts. However, the pH was 6.7 with 10% CO_2 and 6.5 without (difference significant). The pH differed by <0.1 unit among all treatments after aeration of the sprouts at 10°C.

Table 2. Influence of atmosphere composition, duration of CA storage, and aeration after storage on the percentage of salable brussels sprouts.

Storage period (weeks)	Salable buds (%)[z] %O_2/%CO_2 concn 1/0	2/0	1/10	2/10	20/10
At termination of CA storage					
2	97	99	100	100	100
3	94	87	99	99	99
4	71	69	98	98	90
LSD 0.05 (all concn) = 11.3					
SE 0.05 = 3.9					
After 2 or 3 days of aeration at 10°C					
2	93	90	99	99	99
3	87	76	99	97	94
4	55	46	93	93	77
LSD 0.05 (all concn) = 17.5					
SE 0.05 = 6.0					

[z]Each datum is the mean of six observations, averaged across temperatures.

Table 3. Incidence (%) of visible injury and occurrence of extreme bitterness (underlined) in brussels sprouts stored in 0.5% O_2.

Temp. of storage (°C)	Test[z] no.	Duration of storage (wks) 2 a	2 b	3 a	3 b	4 a	4 b	Wt/vol ratio of sprouts[x] (mg/mm³)
		Buds affected (%)						
2.5	1	0	0	0	0	0	0	1.22
	2	15	4	3	3	37	32	0.97
5.0	1	0	0	0	---[w]	0	---	0.78
	2	0	0	0	0	0	12	1.31
7.5	1	0	0	0	0	9	11	0.76
	2	0	5	2	26	6	49	1.49

[z]The tests are equivalent to replications.
[y]Examination a, immediately after removal from indicated storage conditions; b, after 2 or 3 days of aeration at 10°C.
[x]Volume of sprouts was calculated by assuming a sphere with a diameter of (length + width)/2.
[w]No data because of prevalence of decay.

The force required to penetrate cooked sprouts was unaffected by either O_2 or CO_2 concentration. However, sprouts stored at 7.5°C resisted penetration more than sprouts stored at 2.5° or 5° at both examinations (24 vs. 17 N and 26 vs. 19 N, respectively; differences significant). Duration of storage did not affect texture of the sprouts.

CA-induced injuries. A bitter, nauseating flavor developed in the nongreen portion of some sprouts stored with 0.5% O_2 at all temperatures, but it occurred more commonly and more intensely at 5° and 7.5°C than at 2.5° (Table 3). Aeration at 10° did not induce the bitterness if none was evident immediately after storage in 0.5% O_2, but aeration tended to intensify it when it was present after storage. Extreme bitterness was not necessarily accompanied by any visible injury.

Storage in 0.5% O_2 also induced a reddish-tan discoloration of the heart leaves, which normally are yellow (Fig. 5). The injury was present in four of the six tests, and its incidence

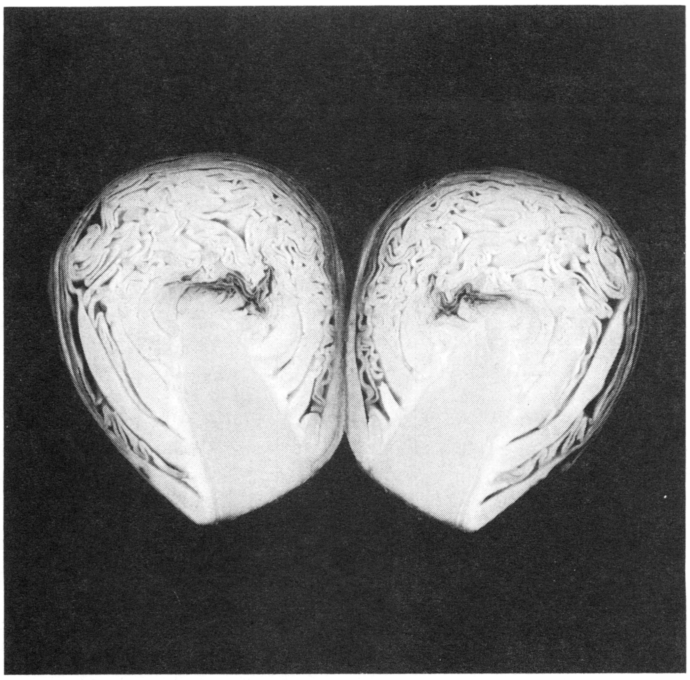

Fig. 5. Necrotic heart leaves due to O_2 deficiency; discoloration is reddish-tan. Injury was induced by storage in 0.5% O_2.

tended to increase with duration of storage, but the influence of storage temperature and subsequent aeration at 10° on symptom expression was equivocal (Table 3). Season of harvest had no influence on occurrence of the discoloration. The green leaves of the sprouts never showed any type of injury attributable to O_2 deficiency.

Injury attributable to excess CO_2 was evident in only one test. Part of the stem internal to the vascular rings was tan in about 25% of the sprouts after they had been stored 19 days at 2.5°C in 1/10. The incidence was about 12% after 2 days of aeration at 10°C. After 26 days, the injury was evident in all samples that had been held with 10% CO_2 (incidence 15% in 20/10 and 38% in 2/10 and 1/10). The incidence doubled during aeration in samples from 20/10 and 1/10, but did not increase in those from 2/10. Thus, aeration does not seem to be a deciding factor in the development of the discoloration.

Discussion

CO_2 production. The reduction in the rate of CO_2 production by storage of the sprouts in low O_2 atmospheres followed a well-documented pattern (4, 25). The relatively low and steady rates observed at 5° and 2.5°C (Fig. 1 B and C) reflect the slow deterioration of the samples at these temperatures. In contrast, the increase in the rate that appeared after about 1 week in samples held in air at 7.5° partly reflected decay development and partly increases associated with general senescence (10).

Part of the initial burst in CO_2 production after transfer of the samples held with 10% CO_2 to air (Fig. 2 A–C) undoubtedly derived from a release of dissolved CO_2. The observation that the rates of CO_2 production during aeration generally were lower the lower the O_2 level that had been combined with 10% CO_2, suggests that the combination more effectively reduced metabolic activity than high CO_2 alone. This trend is reflected in the slightly better retention of salability of lots that had been stored 4 weeks in 1/10 or 2/10 than in 20/10 (Table 2B).

Ethylene production. In general, high ethylene production was associated with low quality. The sharp increases in ethylene evolution at 7.5°C (Fig. 3) accompanied severe yellowing and decay development in all atmospheres but 0.5% O_2. The rapid rise in ethylene production in the latter samples after 23 days of storage may have been associated with the development of low-O_2 injury.

pH and texture. Brussels sprouts responded similarly to broccoli (14) and cauliflower (17) in that lowering the O_2 level had no effect on pH, and high CO_2 resulted in an increase. The latter, however, was considerably smaller than with the other vegetables noted. For them, exposure to high CO_2 resulted in substantial softening of cooked tissue, but had no influence on brussels sprouts.

CA-induced injuries. An examination of the response of brussels sprouts to storage in 0.5% O_2, done briefly earlier (13), is warranted because the expression of the symptoms of injury appears to be related to the different susceptibility of green and nongreen tissue to damage induced by low O_2 levels (12, 15).

Differences in density among sprouts might have influenced the development of the injuries by affecting the diffusion of O_2 to the center of the sprouts. The volume : weight ratio of the sprouts (apparent density) does not provide a clear answer (Table 3). At 2.5°C the looser sprouts exhibited more injury than the denser ones; at 5°, the incidence of visible injury was low in both tests, even though the apparent density differed by a factor of almost 2, whereas the bitterness was present only in the denser sprouts; at 7.5°, visible injury was much more common in the denser lot, but the bitterness affected both about equally. Finally, within a given sample, some dense sprouts had a normal flavor whereas loose ones were bitter.

The problem of diffusion of O_2 to the center was obviated in sprouts that were cut in half. The incidence of visible injury and of bitterness at 2.5° or 5°C was too low (<1%) for meaningful comparisons. At 7.5°, in contrast, 32% whole sprouts were visibly injured but only 2.5% of those that had been halved. The respective values for bitter sprouts were 17% and 0%. Leaching of the bitter compounds, presumably glucosinolates (6), was not involved, because sprouts were cooked with little water. Thus, inadequate diffusion of O_2 into the center may have been the principal factor responsible for development of the injury. However, in one test, the mean weight of whole injured sprouts was nearly the same as for sound ones (14.4 and 15.3 g, respectively). Additionally, the weight of injured sprouts ranged from 8.0 to 19.6 g and that of sound ones from 9.0 to 21.1 g. If inadequate diffusion of O_2 had been a major factor, injury should have been concentrated among the large sprouts.

Our data do not resolve the issue of the factor(s) responsible for the different response of green and nongreen tissue to low-O_2 concentrations. However, they suggest that inadequate diffusion of O_2 does not fully explain the phenomenon. The localization of extreme bitterness in the nongreen center of sprouts may be related to the normal centripetal gradient of glusosinolates in brussels sprouts (G.R. Fenwick, personal communication), but, how very low O_2 accentuates the bitterness is unknown. Thus, regardless of the reasons for the difference in response of green and nongreen tissue, storage of brussels sprouts in 0.5% O_2 is inadvisable.

The injury induced by 10% CO_2 in one test is of interest because this lot of sprouts was harvested immediately following 6 days of unusually high maximum air temperatures (27° to 32°C); all other tests followed periods in which the maxima were between 12° and 25°. However, we do not know whether

the CO_2-induced injury was related to the high preharvest temperatures. Since the discoloration occurred rarely, affected only the stem, and had no adverse effect on the palatibility of the sprouts, it should not be regarded as a deterrent to holding brussels sprouts in a CO_2-enriched atmosphere.

The visual quality of brussels sprouts, which is determined primarily by the degree of greenness of the sprouts and the incidence of decay, can be maintained to a high degree for at least 4 weeks when the sprouts are stored at 2.5°C, whether in normal air or in modified atmospheres. At higher temperatures (5° to 7.5°) storage in 1% to 2% O_2 instead of in air would result in good quality retention, but the addition of 10% CO_2 to the atmosphere would further improve the results. However, since 20/10 yielded almost as favorable results as 1/10 or 2/10, the O_2 level is not as critical as when only low O_2 is used. Under no circumstances should brussels sprouts be held with less than $\approx$ 1% O_2, because 0.5% can induce visible injury and extreme bitterness in the heart leaves.

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