FACTORS AFFECTING THE PROGRESSIVE DEVELOPMENT OF LOW-OXYGEN INJURY IN APPLES

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Controlled laboratory tests have demonstrated significant fruit firmness and titratable acids retention in low-oxygen storage (1.0-1.5% O_2) of McIntosh (3,7,11,12), Cox's Orange Pippin (16,17), Golden Delicious (13), Red Delicious (1,2), Spartan (10,14), Idared (6,9) and other cultivars (7,9). In addition storage of certain cultivars in low oxygen storage may reduce the incidence of scald (2,7,15). Commercial application of low-oxygen technology has introduced an enormous number of variables which may ultimately effect the quality responses and the predisposition of apples to low-oxygen injury. Previous reports have found that cultivar (2,5,6,9,10,11,12,13,14,16,17), fruit maturity (12), fruit origin (5,18) and storage temperature (13,18) may affect the incidence of low-oxygen injury found in individual fruit lots. The present study investigates factors which play in the commercialization of low-oxygen storage and which may influence the susceptibility of fruit to low-oxygen injury.

Cornell Method For Fruit Ethanol Determinations

Quartered apples were sealed into Mason (home canning) jars which had two-part metal screw lids. The dome of each lid was fitted with a rubber serum cap to permit sample withdrawal for head space analysis. One of two procedures were used to analyse the 6-8 apples from each storage lot. a) Each apple was quartered and sealed into a 473 ml (pint) jar - 6 to 8 jars per storage lot. b) 6 to 8 quarters, one from each apple, were composited into a 946 ml (quart) jar - 2 or 3 jars per storage lot. Data in Table 1 show either procedure can be used to obtain average ethanol concentrations. Procedure (a) was used when the range in ethanol concentrations was desired, (b) when a large number of storage lots required analysis and the range data for each lot was not required. Equilibration between head space and tissue ethanol was established in 2-3 hours (Fig. 1).

Standards were prepared by adding ethanol to 250 ml water held in a 500 ml Erlenmeyer flask which was fitted with a standard taper syringe needle adaptor. Standards and apples were held in the lab at 20°C for at least 12 hours before the apples were cut for analysis. Ethanol was quantitatively measured using a FID-GC equipped with a 137 cm x 3 mm diameter Porapak P column held at 120°C. Peak height of ethanol in the Mason jar head space was compared with peak height of ethanol in the head space of the flasks containing ethanol standards (10 and/or 20 mg/100 ml). Previous observations had shown that the ratios of head space concentrations to apple cortical tissue concentrations of ethanol were similar to air/water partition coefficients for ethanol (8).

Kentville Method For Fruit Ethanol Determination

Apple quarters from each of ten fruit maintained at 3°C were weighed and placed in a 1 liter glass jar. The jar was immediately sealed with a metal lid containing a septum to allow for sampling with a hypodermic syringe. The jar containing the apple sectors was held for 1 hour at 25°C after which a 1 ml sample of headspace was removed for injection into the gas chromatograph (Fig. 2).

Headspace ethanol was quantitatively determined using a Varian 3700 gas chromatograph equipped with a flame ionization detector and a 110 cm x 3.2 mm OD stainless steel column packed with Porapak P (80/100 Mesh). Injector, column and detector temperatures were maintained at 110°, 65° and 90°C respectively with a helium carrier gas flow rate of 50 cc/min.

With the above parameters ethanol had a retention time of approximately 4 minutes. Peak height of measurements were compared to a standard curve prepared from a series of ethanol dilutions in water held at 15°C. Separation of acetaldehyde and ethylene were obtained in the same chromatogram.

Results and Discussion

Fruit ethanol levels have been determined to increase as a result of anaerobic respiration resulting from low-oxygen conditions and accumulations of ethanol in apples was positively correlated to the development of low-oxygen injuries (4). Detection of fruit ethanol content has also been used as a index to identify fruit lots which were susceptible to low-oxygen injury (4). McIntosh and Empire apples which were chronically exposed to ethanol levels above 20 mg EtOH/100 g fruit (determined by either Cornell Method or Agriculture Canada Method) developed epidermal blueing and loss of flavour whereas fruit with ethanol contents below this threshold were usually unaffected. However most apple cultivars which were chronically exposed to ethanol levels above 40 mg EtOH/100 g fruit developed internal browning symptoms (may appear as outer cortical browning, diffuse cortical browning, pith browning), low-oxygen scald, off flavours or epidermal cracking. Acute exposures of apples to internal ethanol levels in excess of 120 mg EtOH may result in any of the above low-oxygen injuries.

Empire and McIntosh cultivars demonstrated a negative correlation between the seed count and fruit ethanol levels in certain crop

years (Table 2, S. LaCasse personal communication). Parthenocarpic fruit or fruit with very low seed count (1 to 3 seeds per fruit) are most likely to develop high internal ethanol levels and low-oxygen injury in response to low-oxygen storage conditions and crop years which favour low seed numbers may predispose fruit to a higher than normal incidence of injury. Fruit which have average seed counts greater than 6 are not usually predisposed by this variable to either high ethanol accumulations or low-oxygen injury.

Delays in either the removal of field heat or the establishment of low-oxygen conditions resulted in ethanol accumulation and increased fruit susceptibility to low-oxygen disorders (Fig. 3, Table 3). Delayed field heat removals of 10 days or more resulted in softer fruit and increased ethanol accumulations in Empire apples which had been flushed with N_2 (ca 3% O_2) four days after harvest (Fig. 3). Delays of 20 days prior to O_2 reduction to less than 5% increased the incidence of low-oxygen injury and significantly reduced fruit firmness levels (Table 3). Repeated 1 week exposures of fruit to low-oxygen (1.0% O_2) and ambient air immediately after harvest also increased the susceptibility of individual fruit lots to low-oxygen injury (Table 4). Considering this evidence and commercial tests, re-establishing 1.0% O_2 conditions after a system failure which allowed room O_2 to exceed 1.5% O_2 is not recommended.

Prestorage dips are often applied to fruit which are placed in conventional CA storage. The most common dips applied are those containing antioxidants, $CaCl_2$ or fungicide to control scald, storage pit or senescent disorders and decay respectively. Although low-oxygen storage has been shown to reduce the incidence of scald (2,7,15) and decay (except for Gleosporium spp.) some commercial operations may still consider prestorage dipping of apples destined for low-oxygen storage. McIntosh apples which were dipped in a mixture containing 2000 ppm DPA, 1000 ppm Captan (50 WP), 500 ppm Benlate (50 WP) and 19,000 ppm $CaCl_2$ and stored wet in perforated polyethylene bags in 1-2% O_2 had a significantly higher incidence of low-oxygen injury than the undipped or H_2O dipped controls (Table 5). Only fruit from one of the two orchard blocks demonstrated increased levels of ethanol resulting from the cocktail dip.

To investigate the effects of storage temperature on internal ethanol accumulation and the incidence of low-oxygen injury, McIntosh apples previously held in conventional CA for 6 months were placed in airstreams of 1.0% CO_2 plus 0.3% O_2 and maintained at -0.6, 1.1 and 2.8°C for ten days. In this study, fruit ethanol content was positively correlated with the severity of low-oxygen injury and both fruit ethanol content and low-oxygen injury increased with increased temperatures (Fig. 4). However, McIntosh apples held in either 5% CO_2 plus 3% O_2 or 1.5% CO_2 plus 1.0% O_2 at 0° or 3°C continuously after harvest indicated that low-oxygen injury and low temperature breakdown were increased by storage at 0°, whereas the incidence of senescent disorders were increased by storage in 5% CO_2 or 3% O_2 and/or storage at 3° (Table 6). In no case was low-oxygen injury observed after 90 days of storage but was evident in fruit held at 0° or 3° after 180 days of storage. This observation is similar to that for McIntosh apples held for initial

periods in 0.3% CO_2 plus 1.0% O_2 which were then allowed to readjust to 5% CO_2 plus 3% O_2 at 1.5 month intervals (14, Table 7). However low-oxygen injury in Spartans was observed in fruit stored for only 1.5 months and was substantially higher than that found in McIntosh apples. The incidence of senescent disorders in both cultivars decreased rapidly with short initial periods of 1.0% O_2 storage prior to conventional CA.

Conclusions

1. Apples with low seed numbers (0-3) have the potential to generate injurious levels of ethanol in low-oxygen storage (ca 1.0% O_2) and are susceptible to low-oxygen injury. Commercial fruit selection procedures for low-oxygen storage should identify apple lots with low seed numbers and not store them in low-oxygen atmospheres.

2. Rapid field heat removal and O_2 pulldown is recommended as delays in field heat removals of 10 days or more of delays in the removal of O_2 from the storage of 20 days increased the susceptibility of fruit to low-oxygen injury.

3. Fluctuating O_2 levels should be avoided for low-oxygen storage and should the O_2 climb above 1.5% O_2 it should not be re-established to 1.0%.

4. Prestorage dipping of fruit to be held in low-oxygen storage is not recommended.

5. McIntosh apples held for short initial periods up to 3 months in 1.0% O_2 and subsequently stored inconventional CA were less susceptible to low-oxygen injury and senescent disorders. A bi-regimen CA system may improve fruit quality maintenance and reduce losses due to fruit disorders in long term CA storage.

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Table 1. Headspace analyses of ethanol for six storage lots of McIntosh apples, eight apples per storage lot.

Storage lot	Fruit ethanol content (mg EtOH/100 g fruit)	
	Composite sample 1 L jar containing eight 1/4-apples	Individual apples eight 0.5 L jars 3/4-apple in each jar
A	2.5	2.6
B	4.0	4.3
C	16.9	15.1
D	45.5	45.6
E	115.1	89.0
F	132.7	108.7

Table 2. Relationship of seed content to internal ethanol levels in Empire apples stored in 3% CO_2 plus 2% O_2.

Seed count (Seeds/apple)	Fruit ethanol content (mg ETOH/100 g fruit)
6.6	6
5.4	4
3.3	70

Table 3. Effects of a delayed CA on fruit firmness and incidence of low-oxygen injury in Empire apples stored in 1.0% CO_2 plus 1.5% O_2, 2.8°C until June 1983.

Orchard	Days from harvest to $[O_2] \leq 5.0\%$			
	4		20	
	Fruit firmness (N)	Low oxygen injury	Fruit firmness (N)	Low oxygen injury
A	69.1	0	64.6	+
B	70.9	0	59.7	+
C	67.8	0	61.5	+
D	69.5	0	66.0	+
Mean (SE=1.11)	69.5		62.8	

Table 4. The incidence of low-oxygen injury in response to repeated flushings to 1.0% oxygen cv. McIntosh

Grower	Low Oxygen Injury (%)	
	Single Flushing	Three Flushings
1	0	79
2	5	18
3	5	27
4	7	36
5	10	15
Mean (SE=8.3)	5	35

Table 5. Fruit ethanol accumulation and low-oxygen injury of McIntosh apples stored in 1% CO_2 plus 1-2% O_2, 3°C until April 15, 1985 (n = 5).

Prestorage treatment	Storage Treatment	Orchard Block			
		A		B	
		Fruit ethanol[z] (mg ETOH/100 g fruit)	Low O_2[y] Injury (%)	Fruit ethanol (mg ETOH/100 g fruit)	Low O_2[y] Injury (%)
Cocktail dip[y]	Wet[w]	21.8a[u]	29.4a	83.2a	55.9a
Water dip	Wet	23.4a	1.1b	27.2b	3.5b
Air control	Wet	26.2a	1.2b	24.8b	1.9b
Cocktail dip	Dry[v]	16.2a	3.2b	15.4b	3.2b
Water dip	Dry	24.2a	0.0b	21.0b	2.3b
Air control	Dry	27.2a	0.0b	22.6b	1.0b

[z]EtOH analyses done 3 and 4 days after chamber opened on two 5-apple samples per replication.

[y]Apples held in air (3°C) for one month after room opening prior to sampling. Apples with low oxygen injury had both epidermal blueing and low-oxygen scald.

[x]Consists of 2000 ppm DPA, 1000 ppm Captan (50 WP), 500 ppm Benlate (50 WP) and 19,000 ppm $CaCl_2$.gal.

[w]Stored wet in perforated polyethylene bags.

[v]Fruit dried prior to storage and placed in paper bags.

[u]Mean separation within columns by Duncan's multiple range test, 5% level.

Table 6. Effects of storage temperature, duration, carbon dioxide and oxygen concentration on the incidence of low oxygen and senescent disorders cv. McIntosh.

Storage temperature (°C)	Storage atmosphere (% CO_2 plus % O_2)	Storage duration (days)	Low oxygen injury[z] (%)	Senescent disorder[y] (%)
0	5 + 3	90	0	0
0	5 + 3	180	48[x]	5
0	5 + 3	270	86[x]	8
0	1.5 + 1.0	90	0	0
0	1.5 + 1.0	180	96	0
0	1.5 + 1.0	270	100	0
3	5 + 3	90	0	7
3	5 + 3	180	0	32
3	5 + 3	270	0	43
3	1.5 + 1.0	90	0	0
3	1.5 + 1.0	180	16	0
3	1.5 + 1.0	270	24	0

[z]Epidermal blueing, outer cortical browning, diffuse cortical browning, pith browning and low oxygen scald were included as low-oxygen injury.

[y]Senescent breakdown and senile brown core were included as senescent disorders.

[x]Low temperature breakdown.

Table 7. Effects of initial periods in low-oxygen (0.3% CO_2 plus 1.0% O_2) plus subsequent periods in standard CA on the incidence of low-oxygen injury and senescent disorders in McIntosh and Spartan apples stored in 3° and 0° respectively.

Initial Storage (months in 0.5% CO_2 + 1.0% O_2)	Subsequent Storage[z] (months in standard CA)	Low Oxygen Injury (%)[y]		Senescent Disorder (%)[x]	
		McIntosh	Spartan	McIntosh	Spartan
0	7.5	0	0	18	5
1.5	6.0	0	12	2	1
3.0	4.5	0	28	1	0
4.5	3.0	1	33	0	0
6.0	1.5	2	45	0	0
7.5	0	4	51	0	0

[z]Standard CA for McIntosh 5.0% CO_2 plus 3.0% O_2.
Standard CA for Spartans 3.0% CO_2 plus 2.5% O_2.

[y]Epidermal blueing, outer cortical browning, diffuse cortical browning, pith browning and low oxygen scald were included as low oxygen injury.

[x]Senescent breakdown, and senile brown core were included as senescent disorders.

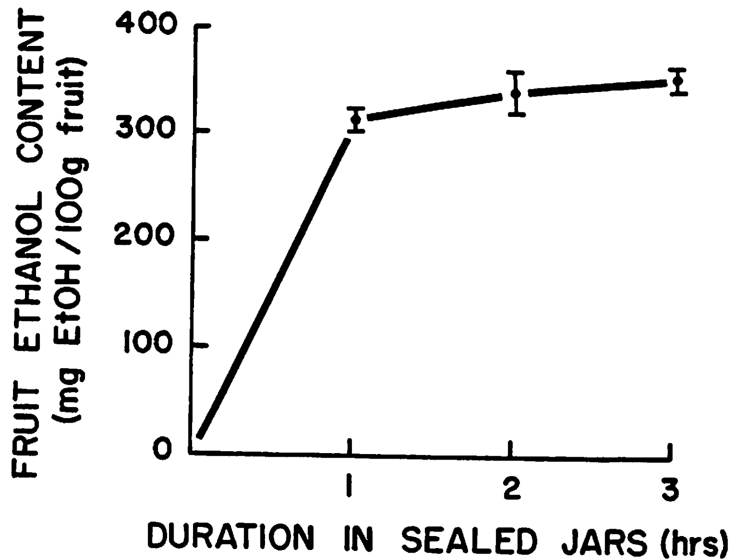

Fig. 1. Accumulation of ethanol in headspace of 0.5 L jars held at 3°C. Four apple quarters per jar, 12 replicate jars. Apples previously held in N2 atmosphere at 21° for 5 days, then equilibrated in air at 3° for 12 hrs before analysis. (Cornell Method)

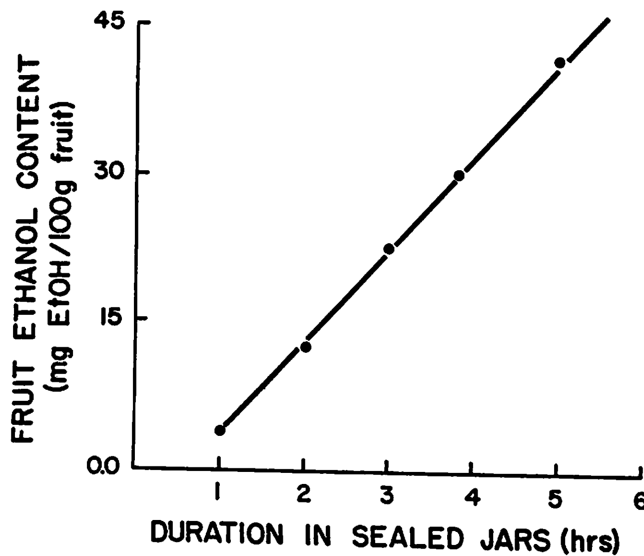

Fig. 2. Accumulation of ethanol in headspace of 1 L jars held at 25°C. Ten apple quarters per jar, 5 replicate jars. Apples removed from storage at 3° sectored, weighed, and placed in jars. (Agriculture Canada method)

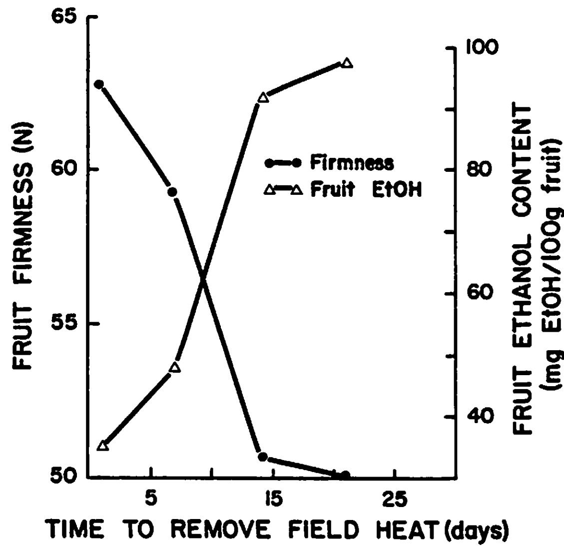

Fig. 3. Effect of cooling rate on flesh firmness and fruit ethanol accumulation Empire stored in Rapid CA (n=2).

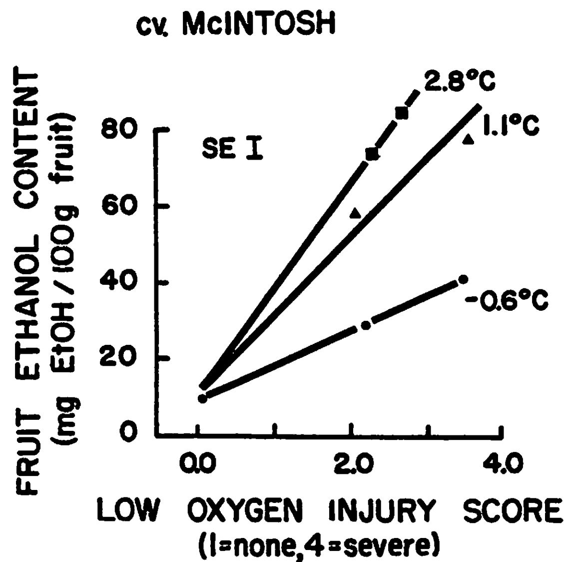

Fig. 4. Relationship of severity of low-oxygen injury to fruit ethanol content (n=3).