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Firmness and concentrations of acetaldehyde, ethyl acetate and ethanol in strawberries stored in controlled and modified atmospheres

Mette Larsen ^{*,1}, Christopher B. Watkins ²

*The Horticulture and Food Research Institute of New Zealand, Mt. Albert Research Centre,
Private Bag 92 169, Auckland, New Zealand*

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Abstract

'Pajaro' strawberries (*Fragaria × ananassa* Duch.) were stored at 0°C in a range of controlled atmosphere (CA) conditions with CO₂ concentrations up to 24%, O₂ concentrations down to 1%, or a combination of 10% CO₂ and 2% O₂. Elevated CO₂ concentrations resulted in firmer fruit, while low O₂ did not affect texture. Off-flavours developed after 3 days of storage at 20% CO₂, but decreased when fruit was subsequently held for 24 h at 20°C. However, off-flavours were persistent after CA storage for 7 days or more. Off-flavours were related to increases in ethyl acetate and ethanol concentrations but not to acetaldehyde. Beneficial atmospheres of close to 10% CO₂ and 2% O₂ resulted in a firmer texture and delayed ripening with no off-flavour development. However, fruit quality was poor when similar atmospheres were developed in modified atmosphere (MA)-producing polythene bags. Rapid imposition of CA resulted in better quality fruit than when MAs around the fruit were developed gradually.

Keywords: *Fragaria × ananassa*; Carbon dioxide; Oxygen; Ripening; Fruit quality; 'Pajaro'; Texture

1. Introduction

Strawberry fruit are highly perishable after harvest, but maintenance of firmness and reduction of decay can be obtained under controlled atmosphere (CA)

* Corresponding author.

¹ Present address: Centre for Food Research, Royal Veterinary and Agricultural University, Thorvaldsensvej 40, 1871 Copenhagen FC, Denmark

² Present address: Department of Fruit and Vegetable Science, Plant Science Building, Cornell University, Ithaca, NY 14853, USA

conditions, particularly high CO₂ treatments (e.g., El-Kazzaz et al., 1983; Ke et al., 1991; Smith, 1992). Some studies have also shown benefits from films which produce modified atmospheres (MAs) (Aharoni and Barkai-Golan, 1987; Shamaila et al., 1992). MA systems in which pallet shrouds around strawberries are injected with CO₂ gas to produce atmospheres of 15–20% are being used commercially to maintain fruit quality (Mitchell, 1992).

Responses of strawberries to CA storage however, can be different according to cultivars and/or growing region (Aharoni and Barkai-Golan, 1987; Browne et al., 1984; Smith and Skog, 1992). Off-flavour development can occur under MA and CA conditions with either low O₂ or high CO₂ (Couey and Wells, 1970; Shaw, 1970; Harris and Harvey, 1973; El-Kazzaz et al., 1983; Browne et al., 1984; Ke et al., 1991; Shamaila et al., 1992). The primary cause of off-flavour appears to be related to accumulation of volatile compounds such as acetaldehyde, ethyl acetate and ethanol caused by anaerobic respiration (Shaw, 1970; Woodward and Topping, 1972; Prasad and Stadelbacher, 1974; Li and Kader, 1989; Ke et al., 1991). Recently, Ke et al. (1991) found strong correlations between off-flavour development and ethanol content, and to a lesser extent, ethyl acetate and acetaldehyde.

Although pallet shroud systems are used commercially for storage of strawberries (Mitchell, 1992), there is little information on the responses of strawberry fruit to smaller-scale MA package systems (Shamaila et al., 1992). Such systems may have greater flexibility for transport of product to markets. As part of a programme to develop MA storage systems for maintaining quality and extending storage life of strawberries, our objective was to examine the influence of a range of CO₂ and O₂ levels on texture and off-flavour development. We then compared the responses of fruit under CA and MA storage.

2. Materials and methods

Strawberry fruit (*Fragaria x ananassa* Duch.) of the cultivar 'Pajaro' were used in this study. 'Pajaro' is the major export cultivar in New Zealand, and is a significant cultivar in both Europe and North America (Hancock and Scott, 1988). Fruit were obtained from a commercial grower in the Auckland area and graded to select intact ripe berries (more than 50% of the surface bright red). Damaged berries, as well as unripe and overripe (very dark red) fruits, were discarded. In one experiment (experiment 1), unripe berries (25–50% red surface) were also selected.

For CA storage experiments, fruit were divided into samples of 1 kg (approximately 50 berries) and stored at 0°C in 80 l chambers, each holding two samples. Precision needle valves were used to mix CO₂, air and N₂ to produce the required atmospheres. The gas mixtures were bubbled through water at 0°C to obtain vapour saturation, and passed through each chamber at 200 ml/min. In all experiments, air-stored fruit were used as control samples. Four experiments were carried out:

(1) Atmospheres of 10 and 20% CO₂ were applied to unripe and ripe berries for 3, 7, and 11 days. At each assessment time, fruit were transferred to 20°C and were analysed after 4 h to allow fruit temperatures to equilibrate, and after 24 h to simulate a shelf life period.

(2) Atmospheres of CO₂ (4, 8, 12, 16, 20 and 24%) and O₂ (1, 2, 4 and 8%), atmospheres, balance N₂, were applied to ripe berries for 14 days. These fruit were analysed only after 4 h at 20°C on removal from CAs.

(3) Atmospheres of 2% O₂ and 10% CO₂ were applied to ripe fruit, either alone or in combination, for 3, 7, 10 and 14 days. Fruit were analysed after 4 or 24 h at 20°C.

(4) Effects of CA and MA storage were compared using an atmosphere of 1.5% O₂ and 11% CO₂ on ripe berries for up to 16 days. For MA storage, fruit were divided into samples of 300 g and sealed in 12 cm × 15 cm 35 μm low-density polythene bags. Control samples were stored in similar bags with the corners cut off to allow air exchange. In the CA system, the atmosphere was created either stepwise, in which the atmosphere was regulated each day to be the same as in the MA system (as indicated in Fig. 3), or rapidly in which the final atmosphere of 1.5% O₂ and 11% CO₂ was applied immediately and maintained during the whole storage period. Fruit were analysed after 4 and 24 h at 20°C. The experiment was repeated once and the data were combined because there were no significant differences between the experiments.

Experiments 1–3 were carried out in the 1991/92 season while experiment 4 was carried out in the 1992/93 season.

Texture was measured on two replicates per sample using a Kramer shear cell in an Instron model 4301 materials testing machine (Instron, Canton, Mass., USA) with a 5 kN load cell. Horizontal slices of 6 mm thickness were cut from the broadest part of 8 fruits selected randomly from each sample, and one quarter of each of these 8 slices were used for one measurement. Two measurements were carried out per replicate. The crosshead speed was 100 mm/min. The firmness was measured as the difference between the maximum force of the recorded peak and the basic force required to move the shear cell. The firmness readings were divided by the total weight of the 8 fruits.

Volatile compounds were determined by headspace analysis. In experiments 1–3, 75 g of quarters of berries were sealed in a 450 ml jar and incubated at 30°C for 90 min. In experiment 4, 300 g fruit was homogenized in a Waring blender for 30 sec (low speed). Duplicate samples of 5 g were transferred to 20 ml vials with sealed septum lids. These samples were stored at –20°C. For subsequent analysis, samples were incubated at 40°C for 20 min. Headspace samples of 1 ml were analysed in a HP5890 gas chromatograph equipped with a DBWAX column (30 m × 0.53 mm × 1 μm) and a FID detector (oven temperature, 50°C; injector, 100°C; detector, 150°C). Acetaldehyde, ethyl acetate and ethanol were detected on the basis of their retention times and quantified by using a range of standards of the compounds (in water). Since the variance increased with concentration, these results were transformed to logarithms prior to statistical analysis (Box et al., 1978, pp. 232–234).

Sensory studies were carried out in experiments 1 and 3 using an untrained panel of eight persons who evaluated the off-flavour on a 0–3 scale: 0 = none; 1 = weak; 2 = medium; and 3 = strong off-flavour. In experiment 4, sensory evaluation was carried out only after 4 days of storage.

All data were tested by analysis of variance procedure with SAS programme package. Significant main effect means were separated by LSD at the 5% level.

3. Results

Levels of rots were negligible in both years of the study and thus no effects of treatments were found.

Effects of 10% and 20% CO₂ on unripe and ripe fruit

In general, unripe berries had firmer texture than the ripe berries, and firmness increased with increasing CO₂ concentrations (Table 1).

Ethanol and ethyl acetate accumulated during storage at 20% CO₂ (Table 1). To a lesser extent acetaldehyde also accumulated at 20% CO₂ but its concentrations also increased in air-stored fruit. After removal from storage, concentrations of the volatiles decreased. Acetaldehyde concentrations were higher in ripe fruit than in unripe fruit, but there was no effect of berry maturity on accumulation of ethyl acetate or ethanol.

The strongest off-flavours were detected in ripe berries stored at 20% CO₂, but unripe berries also developed off-flavours at this atmosphere. This off-flavour decreased after removal to air for 24 h after 3 days storage, but not after 7 or 11 days. Some off-flavour was also detected in the ripe control and 10% CO₂ samples after late removals. The relationships between the off-flavour scores and acetaldehyde, ethyl acetate and ethanol concentrations are shown in Fig. 1. Off-flavour scores were

Table 1

Effects of CO₂ concentration, ripeness, storage time, and removal from storage on the firmness and content of acetaldehyde, ethyl acetate, and ethanol of strawberries. Overall means are shown, and those with different letters within a group are significantly different at the 5% level

| | Firmness (Instron reading/weight) | Acetaldehyde (μ l/kg) | Ethyl acetate (μ l/kg) | Ethanol (μ l/kg) |
|---|--------------------------------------|-------------------------------|--------------------------------|--------------------------|
| <i>% CO₂</i> | | | | |
| 0 | 0.52 c | 3.9 a | 1.9 b | 18.2 b |
| 10 | 0.60 b | 2.8 b | 1.9 b | 12.1 c |
| 20 | 0.69 a | 3.7 a | 27.1 a | 33.4 a |
| <i>Ripeness</i> | | | | |
| Unripe | 0.69 a | 3.1 b | 5.6 a | 19.3 a |
| Ripe | 0.50 b | 3.9 a | 5.3 a | 19.8 a |
| <i>Days of storage</i> | | | | |
| 0 | 0.61a b | 3.0 b | 0.9 c | 14.6 c |
| 3 | 0.65 a | 2.6 c | 3.1 b | 12.4 c |
| 7 | 0.60 b | 4.0 a | 10.5 a | 21.5 b |
| 11 | 0.53 c | 4.5 a | 11.0 a | 33.4 a |
| <i>Hours after removal from storage</i> | | | | |
| 4 | 0.54 b | 3.7 a | 6.5 a | 23.4 a |
| 24 | 0.65 a | 3.1 b | 4.5 b | 15.9 b |

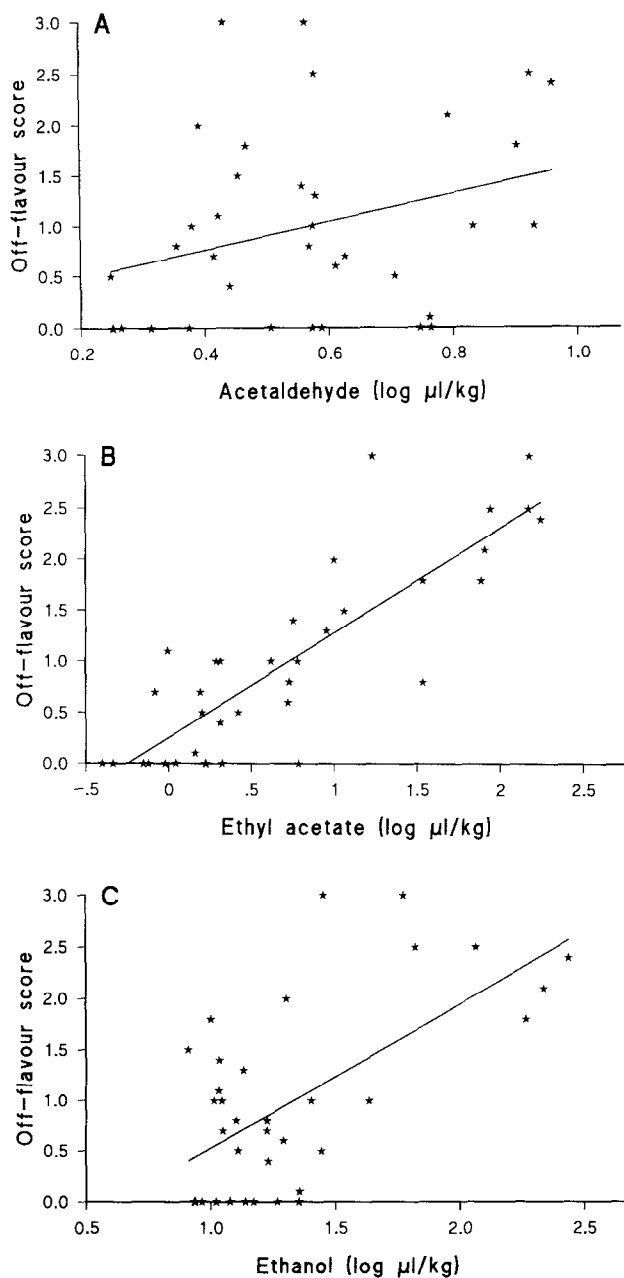


Fig. 1. Relationships between off-flavour scores and content of (A) acetaldehyde; (B) ethyl acetate; and (C) ethanol for ripe and unripe strawberries stored at 0, 10 or 20% CO_2 at 0°C for 3, 7 and 11 days and analysed after 4 h at 20°C . Regressions for off-flavour scores are: $= 1.40 \log(\text{conc}) + 0.20$, $R^2 = 0.09$ for acetaldehyde; $= 1.03 \log(\text{conc}) + 0.25$, $R^2 = 0.72$ for ethyl acetate; and $= 1.42 \log(\text{conc}) - 0.90$, $R^2 = 0.38$ for ethanol.

found to be most highly correlated with ethyl acetate. Some correlation between off-flavour and ethanol was also observed, but not with acetaldehyde.

Effects of CO₂ (0, 4, 8, 12, 16, 20 and 24%) and O₂ (1, 2, 4, and 8%)

Berries stored in all elevated CO₂ concentrations were firmer than those held in air, while firmness was affected only slightly by low O₂ atmospheres (Fig. 2A).

Relative to air control, acetaldehyde concentrations increased at 24% CO₂ (Fig. 2B), ethyl acetate concentrations increased at 16% CO₂, with much higher concentrations at 20 and 24% CO₂ (Fig. 2C), and ethanol concentrations increased at 20 and 24% CO₂ (Fig. 2D) Compared to the changes in volatile concentrations at high CO₂ atmospheres, the responses to low O₂ atmospheres were small (Fig. 2B, C, D).

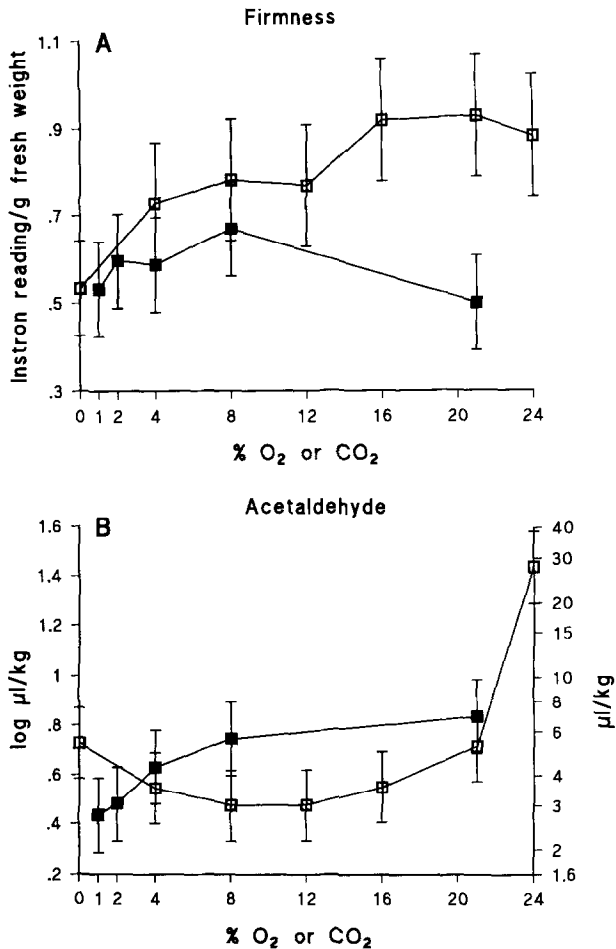


Fig. 2. Firmness (Instron reading/weight) (A) and concentration ($\mu\text{l}/\text{kg}$) of acetaldehyde (B) (means and 95% confidence intervals) for ripe strawberries stored for 14 days at different concentrations of O₂ = ■ or CO₂ = □ and analysed after 4 h at 20°C.

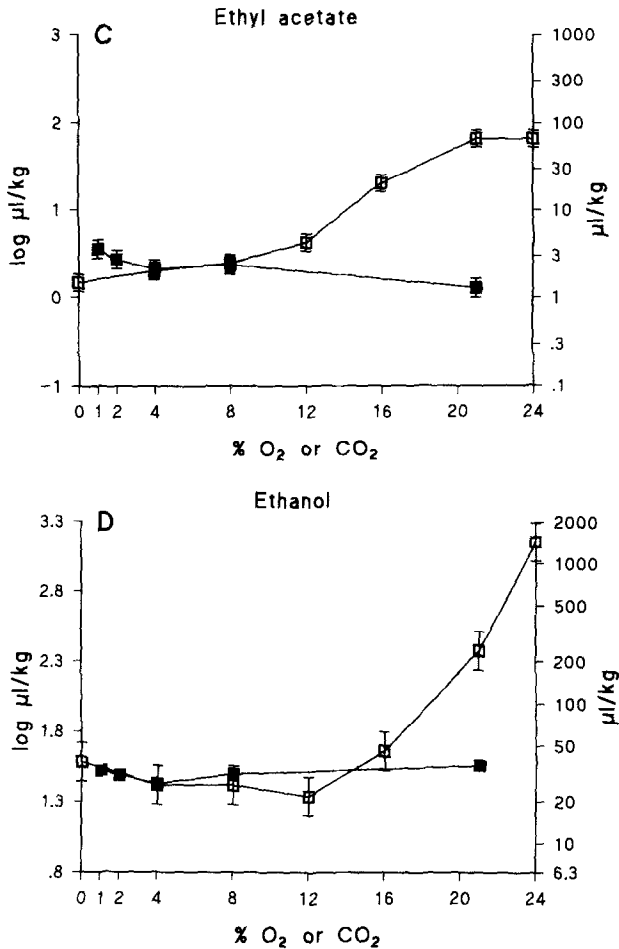


Fig. 2 (continued). Ethyl acetate (C) and ethanol (D) (means and 95% confidence intervals) for ripe strawberries stored for 14 days at different concentrations of O₂ = ■ or CO₂ = □ and analysed after 4 h at 20°C.

Berries from all atmospheres other than 20 and 24% CO₂ had acceptable eating quality.

Effects of 2% O₂ and 10% CO₂ alone or in combination

Fruit stored in 10% CO₂ had the most firm texture while firmness was not affected by low O₂, and there was no interaction between the low O₂ and high CO₂ concentrations tested. Volatile concentrations increased during storage, and the only effects of storage atmospheres were slightly lower concentrations of ethanol and acetaldehyde at elevated CO₂ as well as reduced O₂ (Table 2).

No sensory differences between the samples were detected and in general, only weak off-flavours were detected.

Table 2

Effects of CO₂ and O₂ concentrations, storage time, and removal from storage on the firmness and content of acetaldehyde, ethyl acetate, and ethanol of strawberries. Overall means are shown, and those with different letters within a group are significantly different at the 5% level

| | Firmness (Instron reading/weight) | Acetaldehyde ($\mu\text{l/kg}$) | Ethyl acetate ($\mu\text{l/kg}$) | Ethanol ($\mu\text{l/kg}$) |
|---|--------------------------------------|--------------------------------------|---------------------------------------|---------------------------------|
| <i>Atmosphere</i> | | | | |
| 0% CO ₂ 21% O ₂ | 0.44 b | 4.4 a | 1.8 a | 17.6 a |
| 0% CO ₂ 2% O ₂ | 0.45 b | 2.7 b | 1.0 bc | 13.0 b |
| 10% CO ₂ 19% O ₂ | 0.57 a | 2.5 b | 0.8 c | 9.3 c |
| 10% CO ₂ 2% O ₂ | 0.59 a | 2.7 b | 1.4 a | 10.1 c |
| <i>Days of storage</i> | | | | |
| 0 | 0.41 c | 1.9 d | 0.8 c | 7.4 d |
| 3 | 0.55 a | 2.6 c | 0.7 c | 9.4 c |
| 7 | 0.49 b | 3.1 b | 0.9 bc | 11.7 b |
| 10 | 0.50 b | 3.2 b | 1.3 b | 14.2 b |
| 14 | 0.52 ab | 4.7 a | 2.8 a | 19.8 a |
| <i>Hours after removal from storage</i> | | | | |
| 4 | 0.53 a | 2.7 b | 1.1 a | 11.1 b |
| 24 | 0.49 b | 3.3 a | 1.3 a | 12.7 a |

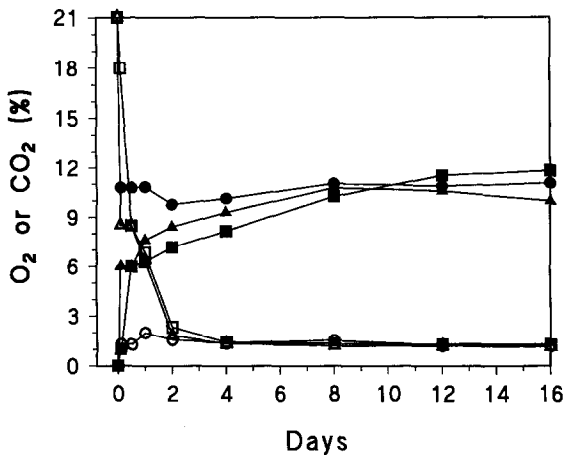


Fig. 3. Concentrations of CO₂ (closed symbols) and O₂ (open symbols) in modified atmosphere packages (■, □); rapid CA storage (○, ●); and stepwise modification of CAs (△, ▲).

Effects of 1.5% O₂ and 11% CO₂ in CA and MA systems

Changes in atmosphere during development of MAs, and in rapid and stepwise CA storage are shown in Fig. 3. Oxygen concentrations had fallen to about 1.5% by 4 days and those of CO₂ had risen to about 11% by 8 days.

Fruit stored under rapid CA conditions were firmest. Stepwise CA and MA conditions also had some effect on firmness, and there were no differences between

Table 3

Effects of storage conditions, storage time, and removal from storage on the firmness and concentration of acetaldehyde, ethyl acetate, and ethanol of strawberries. Overall means are shown, and those with different letters within a group are not significantly different at the 5% level

| | Firmness (Instron reading/weight) | Acetaldehyde ($\mu\text{l}/\text{kg}$) | Ethyl acetate ($\mu\text{l}/\text{kg}$) | Ethanol ($\mu\text{l}/\text{kg}$) |
|---|--------------------------------------|---|--|--|
| <i>Storage conditions</i> | | | | |
| Control | 0.37 c | 1.6 b | 1.4 e | 10.6 b |
| MA | 0.44 b | 6.3 a | 117.5 a | 144.0 a |
| CA-control | 0.36 c | 1.9 b | 1.9 d | 11.1 b |
| Stepwise CA | 0.44 b | 1.2 c | 2.9 c | 9.9 b |
| Rapid CA | 0.48 a | 1.5 b | 5.2 b | 9.7 b |
| <i>Days of storage</i> | | | | |
| 0 | 0.33 c | 1.4 c | 0.7 e | 6.8 e |
| 4 | 0.41 b | 1.2 c | 2.5 d | 9.7 d |
| 8 | 0.44 a | 2.1 b | 5.9 c | 16.6 c |
| 12 | 0.41 b | 2.2 b | 8.1 b | 22.0 b |
| 16 | 0.44 a | 4.5 a | 14.5 a | 41.5 a |
| <i>Hours after removal from storage</i> | | | | |
| 4 | 0.41 a | 2.4 a | 5.9 a | 22.0 a |
| 24 | 0.41 a | 1.7 b | 4.3 b | 12.5 b |

the two control samples (Table 3). Ethyl acetate and ethanol, and to a lesser extent acetaldehyde, accumulated to very high levels during MA storage, while the concentration of these volatiles was similar to the control for the CA stored samples. Generally, volatile concentrations increased during storage and decreased after removal from storage (Table 3). Sensory evaluation after 4 days of storage indicated that MA-stored fruit had a very strong off-flavour while all other samples had no off-flavours.

4. Discussion

Texture

Of the quality factors investigated in our experiments the most marked response was the maintenance of texture under elevated CO_2 atmospheres, even at a concentration of 4%. Beneficial effects on texture with 6% CO_2 have been reported (Smith, 1992), but we have not been able to find reports on texture at lower CO_2 concentrations. Further research is needed to determine the minimum CO_2 concentration that the effect on texture occurs. In our experiments, only slight effects of low O_2 were detected, and we found no indications of interactive effects of low O_2 and high CO_2 . Texture effects of low O_2 have been found by Li and Kader (1989) and Ke et al. (1991), but the O_2 levels of 1% or less were lower than used in our study.

Volatiles

Acetaldehyde is regarded as one of the most prevalent products of anaerobic respiration. In the present study, however, acetaldehyde accumulated only in fruit

stored at 24% CO₂ or in MA storage, while other CA conditions did not result in significantly higher concentrations of acetaldehyde than found in air-stored fruit. In contrast, after 14 days of storage, the concentration of acetaldehyde in CA-stored fruit was generally lower than that of air-stored fruit. Accumulation of acetaldehyde in air-stored fruit may be a result of the physiological breakdown caused by over-ripeness (Smagula and Bramlage, 1977).

While acetaldehyde probably accumulates because of over-ripeness, the present results show that ethyl acetate and ethanol may be regarded as the most important volatiles caused by anaerobic respiration. Acetaldehyde is a product of anaerobic respiration, but it may be reduced to ethanol and react further to form ethyl acetate. These reactions may be more likely to occur under anaerobic conditions than in overripe fruit. Li and Kader (1989) also found that ethanol concentrations increased more than acetaldehyde during CA storage while Ke et al. (1991) have reported major increases of all three volatiles under such conditions.

Relations between volatile compounds and off-flavour development

In the present study, ethyl acetate was related best to off-flavours as indicated by the high R² value, and the higher level of this compound persisted with off-flavour after removal from CA conditions. This high CO₂-induced off-flavour also had a characteristic ethyl acetate note. We also detected another weaker off-flavour due to over-ripeness in air-stored fruit. This off-flavour may be partly explained by the acetaldehyde content.

Relationships between off-flavour scores and ethyl acetate content (Fig. 1B) suggested that the off-flavour of a sample might be estimated from the ethyl acetate concentration. Thus an ethyl acetate concentration of 5 µl/kg is equivalent to an off-flavour score of 1 (weak off-flavour), and 50 µl/kg is equivalent to an off-flavour score of 2 (medium off-flavour). When this relationship was applied to data obtained in experiment 2, the off-flavour score of fruit stored at 16% CO₂ should have been approximately 1.6, but this fruit was found to have an acceptable flavour. In experiment 3, only weak off-flavours were predicted from the ethyl acetate concentrations which was in accordance with the sensory evaluation. In experiment 4, an off-flavour score of 2.4 was predicted for the MA treatment while low scores were predicted for the remaining treatments. These results indicate that the relationships between ethyl acetate and off-flavour scores found in the first experiment may generally be usable for 'Pajaro' strawberries, and that ethyl acetate levels up to 5–10 µl/kg may be regarded as acceptable. Flavour, however, is produced by many different volatile compounds and although ethyl acetate is a good indicator of off-flavour, this compound is not the sole compound responsible for off-flavour.

Ke et al. (1991) also studied the relations between off-flavour scores and acetaldehyde, ethyl acetate, and ethanol. They found high correlations for all compounds, and predicted acceptable levels of the three compounds of 8.1, 63, and 23 µl/l, respectively. The level for ethyl acetate is much higher than that found in the present study, and differences may be related to effects of cultivar.

Differences between CA and MA storage

Significant differences in fruit quality result from use of flow-through CA systems and slower development of atmospheres inside MA bags. The same atmospheres which give good results on texture under CA conditions do not necessarily work under MA systems.

Rapid CA was the most effective treatment, while there was no difference between MA and stepwise CA (Table 3). This result may be caused by a 'shock-effect' of CO₂ when the fruit is treated rapidly with high gas concentration. However, there were also large differences in accumulation of volatiles and development of off-flavours between MA- and both types of CA-stored fruit. A possible explanation could be that volatile compounds such as acetaldehyde accumulate in the polythene bags but that they are removed by gas movement in the CA system. This view was supported by measurement of high concentrations of volatiles in the bags (data not shown) and in the fruit (Table 3). We attempted to reduce volatile concentrations around the fruit by packing 200 g strawberries with 50 g activated carbon in polythene bags, but after 2 weeks of storage no differences in quality between this fruit and fruit stored under normal MA conditions were found (data not shown). Apparently, activated carbon was not sufficiently able to absorb the volatile compounds responsible for off-flavour development. Accumulation of volatiles in MA packages is also a limitation in other fruit systems, e.g. persimmons (Ben-Arie et al., 1991).

Differences in responses of strawberries to high CO₂ concentrations in pallet shroud and other MA-producing systems, which may be related to cultivar and/or growing region, are apparent (Browne et al., 1984; Aharoni and Barkai-Golan, 1987). It is not known if these differences are also shown in CA storage, although varying cultivar responses have been demonstrated (Smith and Skog, 1992). A study of responses to CA and MA in relation to cultivar differences is warranted.

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