Effect of modified atmosphere packaging (MAP) on soft fruit quality

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Soft fruits such as strawberries and raspberries, have a very short post-harvest shelf-life which is exacerbated by infection with grey mould caused by the fungus *Botrytis cinerea* (Fig. 1). There are significant commercial gains to be made by minimising such deteriorative losses in both retail outlets and in the home. Strategies to extend the shelf-life of fruit include traditional breeding, genetic manipulation and post-harvest treatment of the fruit or its storage environment. Thus far, traditional breeding of strawberry and raspberry has had only limited success in extending shelf-life. Genetic engineering is an attractive prospect as the concept is already proven for tomato and melon, where restricting ethylene production improves fruit longevity in store. As yet, such technology is unproven for raspberries and strawberries. Although ethylene is also likely to play a key role in regulating raspberry quality traits[1], mechanisms controlling strawberry post-harvest quality are less obvious.

Figure 1 Strawberry infected with *Botrytis cinerea*.

Whilst the biological processes underpinning such processes are being elucidated, other options are available to the commercial sector. These include post-harvest treatments such as coating the fruit with a protective film e.g. chitosan, or storage of fruit under a controlled or modified atmosphere to minimise deteriorative losses.

In retail outlets, strawberries and raspberries are generally stored in closed, perforated-top containers. Within these containers, the fruits continue to respire the 'trapped' air until the CO_2 concentration rapidly approaches the critical 10-15% level necessary to inhibit *Botrytis* growth. However, depending on the state of the fruit at harvest, the time taken to pack and the storage temperature, *Botrytis* infection may be significant before the CO_2 concentration has reached the critical level required to suppress fungal growth. An alternative approach is to flush the package with known gas mixtures to provide, directly, a modified storage atmosphere and/or to use impermeable or selectively permeable packaging films to maintain the best environment for as long as possible. The approach generally involves gas mixes confined to 5-15% CO_2, 2-5% O_2, with N_2 as the remainder. The elevated CO_2 concentration at packaging generally rises during the first 12 h and inhibits *Botrytis* growth. However, one drawback is that the continued presence of an elevated CO_2 environment induces a concomitant decrease in the pH of the fruit, leading to a deleterious 'fizzy' or sharp taste. This can be alleviated by employing one of two methods: (1) A novel modified atmosphere packaging (MAP) gas system with O_2 as the dominant gas; and (2) A semi-permeable membrane which facilitates the diffusion of moisture and gases establishing an equilibrium state which, once optimised, retards deterioration of the fruit. At the SCRI, these strategies have been assessed for the possibility of extending storage life of both

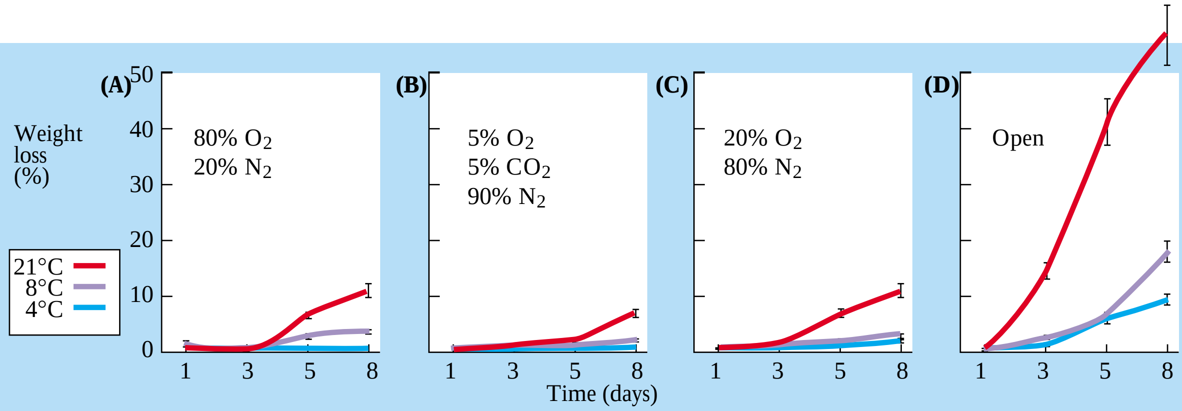

Figure 2 Weight loss of strawberries packaged in (A) 80% O_2:20% N_2; (B) 5% O_2:5% CO_2:90% N_2; (C) 20% O_2:80% N_2; and (D) in the absence of packaging.

strawberries (high O_2 approach) and raspberries (semi-permeable membrane approach).

Effect of elevated CO_2 or O_2 on strawberry quality
Strawberries have previously been the subject of MAP studies largely relying on the use of elevated CO_2 and reduced O_2 levels[2]. These have met with limited success, the main problem being the progressive increase in CO_2 concentration leading to increased acidity in the fruit. The advent of MAP approaches using elevated O_2, rather than elevated CO_2, appears to alleviate this problem with the added advantage of retaining fruit firmness. This system has been used successfully with meat and, more recently, with cut vegetables[3]. The use of O_2 at concentrations much greater than that present in air (50% as compared with 20%), prevents microbial spoilage by dramatically reducing the activity and proliferation of lower organisms. Its use, therefore, should help inhibit the growth of microbes unaffected by ≤20% CO_2[4]. Little is known about the biochemical changes induced in fruit by modified atmospheres and, particularly, the effects of elevated O_2. Here we report the results of an investigation on the impact of MAP on cell wall hydrolase enzymes, which may have a role in regulating fruit firmness, and antioxidant status, an increasingly important parameter which gauges the potential of the fruit to quench free radicals.

Experimental approach Fruit of cultivar Elsanta were picked from field-grown crops at the red-ripe stage, stored at 4°C for 1 h and packaged in containers held in impermeable polypropylene bags (OPP1 film). Packaging was carried out with an industrial packager (CVP Systems). The gas mixtures used were: (a) 20%O_2:80% N_2 (air-control), (b) 80% O_2:0% N_2 (elevated O_2) and (c) 5 % O_2:5% CO_2:90% N_2 (elevated CO_2). The packaging procedure involved two cycles of ambient gas removal/packaging-gas flushing prior to sealing of the packaging film. The packaged fruit were stored at 4°C, 8°C and 21°C and sampled at set periods over 11 days. Non-packaged fruit were also stored and sampled for comparative purposes.

Effects on quality parameters There was little to distinguish between the different packaging gases with respect to fruit weight loss. All gas mixtures were more effective at loss reduction than open packaging (Fig. 2). Not surprisingly, weight loss was inhibited at the lower storage temperatures. An examination of the concentrations of gases within the packaging revealed an increase in CO_2 (to a threshold of *c.* 50-60%) and a decrease in O_2 over the 11 day storage period, irrespective of the original gas mixture used (Fig. 3a-c). However, within the important initial stages of storage, CO_2 reached the level known to inhibit *Botrytis* infection (>10% CO_2) when fruit was stored under elevated CO_2. This was true for storage at 4°C and 8°C. Not surprisingly storage at 21°C rapidly generated CO_2 concentrations which impaired flavour by raising fruit acidity. Storage under air at 4°C and 8°C resulted in a gradual production of CO_2 but the inhibitory CO_2 level for *Botrytis* was only reached after 4-5 days, too late to be effective as a storage regime. Fruit packaged under elevated O_2 experienced a steady reduction in O_2 levels at 4°C and 8°C but these remained above the important 50% concentration over the first 4 days of storage, thereby inhibiting microbial growth[3].

Activities of cell wall hydrolysing enzymes The enzymes β-Galactosidase (β-Gal), arabinofuranosidase (Arab) and cellulase (Cx) have previously been identified as important in the soft fruit ripening processes[5], with β-Gal and Arab thought to be responsible for trimming the constitutive arabinogalactan-substituted

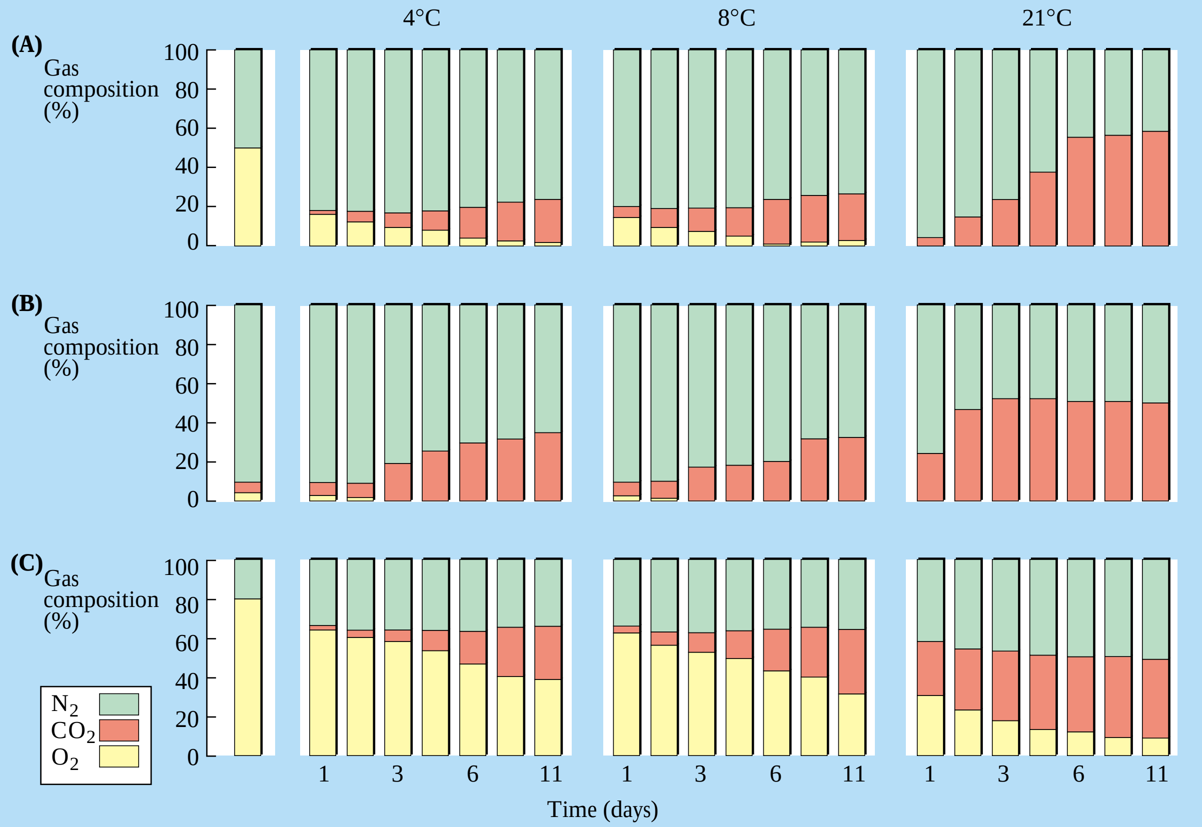

Figure 3 Gas composition of strawberries packaged in (A) 20% O_2:80% N_2; (B) 5% O_2:5% CO_2:90% N_2; and (C) 80% O_2:20% N_2. (C - control cylinder gas. Time t = 0).

rhamnogalacturonic acids prior to extensive pectin solubilisation. There was little difference in β-Gal and Arab activities between the gaseous storage regimes during the first 5 days of storage at 4°C and 8°C. However, enzyme activities were elevated during storage at 21°C (data not shown).

Cx activities varied with storage temperature and gaseous environment (Fig. 4). In all cases, activities were greatest in fruit stored at 21°C. Both the air- and elevated CO_2-packaged fruit showed progressive increases in Cx activity from 4°C to 8°C, more so under air. However, elevated O_2-packaged fruit showed no difference in Cx between the two low-storage temperatures but activity was again elevated at 21°C. In fact, there is no significant change in Cx activity throughout the period of storage at 4°C or

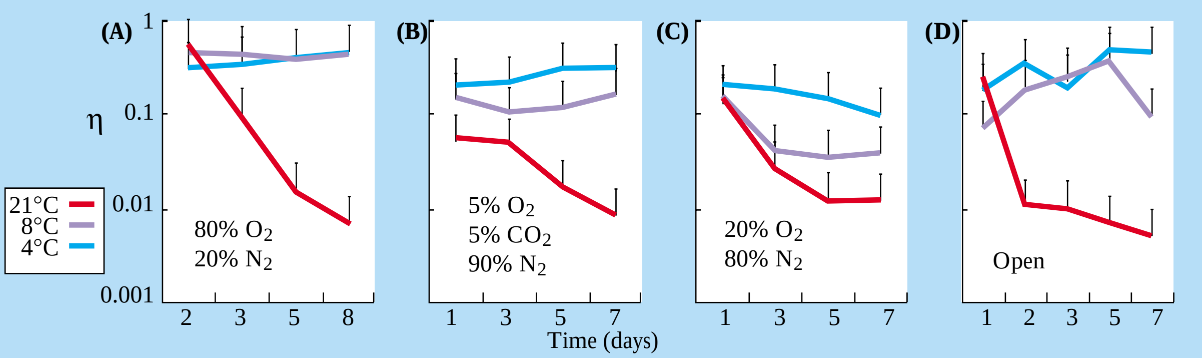

Figure 4 The effect of packaging gas on cellulase activity. NB. Cellulase activity is inversely proportional to viscosity in this assay.

Time (days)	Air 4.0	Air 8.0	Air 21.0	Elevated CO_2 4.0	Elevated CO_2 8.0	Elevated CO_2 21.0	Elevated O_2 4.0	Elevated O_2 8.0	Elevated O_2 21.0
1.0	*****	*****	***	*****	*****	***	*****	*****	***
2.0	*****	*****	*	*****	*****	*	*****	*****	**
3.0	*****	****	*	****	****	*	*****	*****	**/*
4.0	*****	****	*	****	***	*	*****	*****	*
6.0	****	****	n.a.	***	**	n.a.	*****	****	*
8.0	****	***	n.a.	***	**	n.a.	*****	***	n.a.
11.0	***	**	n.a.	**	**	n.a.	****	*	n.a.
30.0	***/**	***/**	n.a.	*	*	n.a.	****	*	n.a.

***** → * : Decreasing numbers of stars indicates a decreasing degree of firmness. n.a. - this fruit had essentially disintegrated and was unsuitable for testing.

Table 1 Effect of MAP regimes on subjectively scored strawberry fruit firmness.

8°C under elevated O_2. This is important since the expression of Cx genes increases substantially in ripening strawberry fruit (Giorgio Casadoro, pers. comm.). Subjective firmness measurements of the packaged fruit indicated that, at all temperatures and sampling periods, the fruit stored in high O_2 were the firmest (Table 1).

Fruit antioxidant status A parameter becoming increasingly important with respect to fruit and vegetables is antioxidant status - the ability to inhibit the formation of free radicals. These highly reactive moieties have been repeatedly linked with many degenerative diseases in humans. The use of elevated CO_2 as the packaging gas reduced the overall antioxidative capacity, in particular during the important initial storage period (Fig. 5). The antioxidant status of air-packaged fruit initially decreased but subsequently increased. This may be due to microbial fermentation reactions producing acetaldehyde which would artificially raise antioxidant status. Fruit stored under elevated O_2 exhibited good antioxidative capacity over the first 4 days of storage but this declined with prolonged storage, possibly due to O_2-promoted oxidation of the constitutive anthocyanins and phenolics. There was visible evidence of mild bleaching in skin colour accompanying prolonged storage (data not shown). However, over the all-important first four days of storage the effect of elevated O_2 on antioxidative status was minimal.

The impact of elevated O_2 packaging on fruit longevity is clearly seen in Figure 6 (unpackaged fruit are shown for comparison). Strawberries were stored for 60 days following packaging under high O_2. On opening, the fruit were definitely edible, whilst lacking the 'tartness' associated with fresh fruit. This is clearly an extreme exercise but demonstrates the efficacy of elevated O_2 storage.

Modified atmosphere packaging of raspberry fruit A major advancement within the MAP industry has been the development of P-Plus™ packaging film. This film allows the diffusion of moisture and has a gaseous permeability that can be varied to suit the high rates of moisture loss and respiration that are

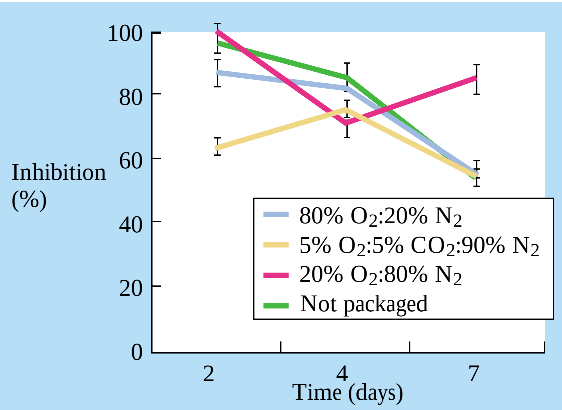

Figure 5 The effect of packaging gas on strawberry antioxidant status. Both packaged and non-packaged fruit were stored at 8°C

Figure 6 Strawberry packaged in 80% O_2: 20% N_2 after 60 days and a control open container.

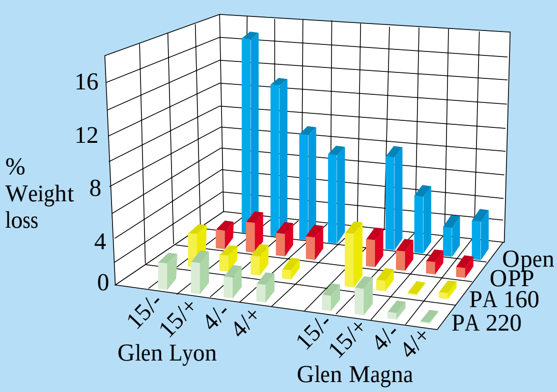

Figure 7 Effect of modified atmosphere packaging (no film (open), OPP, PA160, PA220), temperature (15 or 4°C) and ± Ethysorb™ on the weight loss of ripe raspberry fruits

characteristic of harvested raspberry fruit. The impact of packaging two varieties of raspberries, Glen Lyon and Glen Magna, with semi-permeable films on quality and shelf-life has been determined together with the effects of Ethysorb™, an ethylene 'scrubber'. Ethylene has been implicated as an important factor in determining post-harvest quality in raspberry fruit[5].

Experimental approach Freshly picked fruit were placed in plastic containers which were either left uncovered or sealed with impermeable (OPP) or semi-permeable films with high (PA220) and low (PA160) gas permeability. The fruit was stored at either 4°C or 15°C. Half of the fruit packages included an Ethysorb™ sachet. Shelf-life was defined as that period of time between harvest and the point when the fruit was no longer marketable either due to the development of off-odours, off-flavours or unacceptable appearance. Fruit was scored for incidence of *Botrytis* and changes in taste, colour, firmness and weight loss.

Effects on flavour and colour Packaging made no significant difference to the taste scores. Colour was maintained best at 4°C with the packaged fruit scoring higher than those left unpackaged (data not shown).

Firmness and weight loss The presence of Ethysorb™ helped retard softening during storage, particularly at 4°C. This supports our previous findings[5]. The combined benefits of Ethysorb™ and low storage temperature on firmness were most apparent for fruit stored using the less permeable OPP and PA160 packaging films. It is possible that the moisture retention abilities of these films contributed to prolonged fruit firmness. The most marked advantage of using packaging films was to restrict weight loss (Fig. 7), particularly at the higher storage temperature.

Resistance to *Botrytis* Overall packaging reduced the incidence of *Botrytis* infection compared to the unpackaged fruit.

If any one of the above quality parameters fell below a specified level the fruit would not be acceptable for sale. It was therefore possible to classify this data according to a consumer 'predicted acceptability rating' (PAR). The criteria for classification are defined in Table 2 where 'above-PAR' and 'PAR' represent good- or acceptable-quality produce, respectively. A rating of 'below-PAR' would not be marketable. Table 3 classifies each treatment according to those attributes that were 'below-PAR' and identifies the successful packaging treatments. It is evident that storage at elevated temperature reduces fruit quality regardless of variety. The presence of Ethysorb™ was beneficial at both storage temperatures and resulted in more quality parameters attaining the desired standard.

In general, a reduction in packaging film permeability was accompanied by an increase in the acceptability of the corresponding fruit. Under some storage conditions the incidence of *Botrytis* was the only parameter on which the packaging film failed. The only acceptable ratings were furnished by fruit packaged under PA160 film.

Summary There is clearly considerable scope to extend the quality and shelf-life of perishable fruits such as strawberries and raspberries by developing and

Taste (Score)	Colour (Score)	Firmness (%)	Weight Loss (%)	Mould (%)	PAR
Sweet (4-5)	Red (5)	81 - 100	0 - 2	0 - 1	Good (above-PAR))
Sweet/Sour (3)	Dark Red (3-4)	61 - 80	3 - 5	2 - 3	Acceptable (PAR)
Sour/Acid (1-2)	Very Dark Red (1-2)	< 60	> 5	> 4	Not acceptable (below-PAR)

For each treatment fruit-quality attributes were assessed and assigned one of three character descriptions. Each description corresponds to a 'predicted acceptability rating' (PAR).

Table 2 Classification of packaged fruit on the basis of taste, colour, firmness, weight-loss and incidence of *Botrytis*.

Variety	Storage Temp.(°C)	Ethysorb™	Film type None	OPP	PA160	PA220
Glen Lyon	15	-	C, F, W, B	F, B	T, B	T, C, F, B
		+	C, F, W, B	B	T, B	T, C, B
	4	-	W, B	B	B	B
		+	W, B	B	*	B
Glen Magna	15	-	C, W, B	B	C	C, F, B
		+	B	C	C	C, B
	4	-	C, B	B	B	T
		+	W, B	B	*	B

Each letter denotes the quality attribute for which a treatment failed to be acceptable, and is defined as: T - taste, C - colour, F - firmness, W - weight loss and B - Botrytis. Packaged fruit which had a high quality after storage is indicated '*'.

Table 3 Packaging treatments classified according to failed quality attributes.

evolving modified atmosphere packaging strategies that fully exploit the potential of both packaging films and gaseous storage regimes at harvest and during the storage period. The ability to extend shelf life has clear commercial implications but also offers unique opportunities to manipulate and dissect the control of processes which regulate ripening and post-harvest performance. This is highly complementary to existing strategies based on GM crops.

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