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Benefits of Combined Treatment Approaches to Maintaining Fruit and Vegetable Quality

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ABSTRACT

The use of combined treatments to manage fruit and vegetable quality has been successfully applied in commercial practice, particularly for apples. This historical success is well documented. Unfortunately, the extensive use of combined treatments has not been adopted as a general principle to optimize produce quality, shelf life and safety. The thesis of this review is to discuss the basis for the effectiveness of combined treatment approaches and also discuss potential combined treatments that could significantly improve quality, shelf life and safety of a wide range of fruit and vegetable products. The use of molecular tools in the evaluation of synergistic physiological responses to combined treatments is also discussed.

Keywords: molecular tools, postharvest treatments, quality, stress response

Abbreviations: CA, controlled atmosphere; **$CaCl_2$**, calcium chloride; **MA**, modified atmosphere; **$NaClO_2$**, sodium chlorite

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INTRODUCTION

The use of the combined treatment approach has been a commercial postharvest reality for many decades in the form of low temperature storage with the co-application of low O_2 and high CO_2 atmospheres. This approach has been labelled as controlled atmosphere (CA) storage and hence the concept of combined treatments is not necessarily associated with its application. The great success of long term apple storage using low temperatures in combination with CA (Ben-Yehoshua *et al*. 2005) provides strong support to the concept of applying multiple treatments to fruits and vegetable to enhance quality retention. Similar successes have been shown for the use of low temperature shipping temperatures combined with modified atmosphere packaging of other commodities (Ben-Yehoshua *et al*. 2005). Underlying reasons for these successes are based on the ability of each of the treatments, in the combination, to provide complementary or synergistic activities in controlling quality decline. A brief discussion of the development of low temperatures and CA for apples will form the basis for framing the discussion of options for combined treatments in other fruits and vegetables. More recent advances involving combinations that include physical treatments, ripening control and biocontrol agents, anti-microbial compounds and/or anti-browning formulations in modified atmosphere systems will then be examined for commodities other than apples. The discussion of the advances made with these other commodities, using a combined treatments approach, will provide a mechanistic background by which to stimulate further research in this area of study.

BASES OF CONTROLLED AND MODIFIED ATMOSPHERE SUCCESS

The application of low temperature to control respiration and metabolism has been a well known principle for over half a century (Morris and Brady 2005). Lowering of temperature generally slows metabolic processes in fruit and vegetable tissues and usually this relationship is expressed as a Q_{10} value. A Q_{10} value is defined as the fold change in a specific metabolic process in response to a 10°C change in temperature (Saltveit 2005). However, not all processes slow with the same Q_{10} value (Burg and Thimann 1959) and so imbalances in metabolic processes can arise when fruit is cooled to low temperatures, leading to accumulations of metabolites and consequent quality disorders (Hulme *et al*. 1964). More recently, it has been determined that shifts in temperature can lead to differential gene expression which is also associated with differential metabolite accumulations (Iba 2002; Kaplan *et al*. 2007). Hence, not only can low temperature affect enzyme activities, it also can affect the levels of *de novo* synthesis of enzymes involved in metabolite utilization and inter-conversion. Low temperature storage is also important to controlling the growth of plant pathogenic micro-organisms (Sommer 1992) as well as those which could pose food safety risks to consumers (Tompkin 1973). In general, low temperature is considered

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to be the first "pillar" of good postharvest practice, primarily because it is effective in slowing plant ripening and metabolism while also controlling microbial growth and spoilage of fruits and vegetables (Kader 1980; Mitchell 1992).

CA provides two potential effects on metabolism and ripening; 1) low O_2 can provide significant reductions in the rates for oxygen-dependent processes, and 2) high CO_2 can have a multitude of effects on the rates of a wide range of metabolic processes. In apple, low O_2 and high CO_2 are generally used in tandem to achieve the desired results on quality retention and ripening inhibition (Fidler and North 1967a; Kader 1980). Specifically, application of low O_2 atmospheres can inhibit such varied activities such as respiration, ethylene production and action, browning reactions and respiration-associated flavour volatile production (Knee 1973, 1980). While application of low O_2 atmospheres is associated with respiratory inhibition, the affinity of cyctochrome c oxidase, the terminal oxidase in respiration is very high and inhibition of the respiratory process would require lowering O_2 levels to 0.1 kPa or less (Burton 1978; Knee 1973). However, there are a number of low-oxygen-affinity enzymes which are inhibited at O_2 levels used in CA storage and they are; polyphenol oxidase, b-type cytochromes, ascorbic acid oxidase and glycolic acid oxidase (Burton 1978). In addition, Burton (1978) concluded that a part of the mechanism by which low O_2 levels inhibited climacteric respiratory rise was mediated by the physical fact that insufficient levels of O_2 can diffuse into the fruit tissue to sustain high levels of consumption characteristic for climacteric respiration. Therefore, the inhibition is not a direct consequence of the low O_2 levels commonly used in the storage, yet those levels are often interpreted as being directly limiting on respiration. The enzymes involved with ethylene production are known to have a low affinity to O_2 and atmospheres containing 1.5-2.0 kPa O_2 have been demonstrated to decrease ethylene production by 50% in apples (Burg and Thimann 1959). Low O_2, through its effect on inhibition of ripening also has been attributed with delaying the onset of fruit tissue susceptibility to fungal pathogen invasion (Burton 1978). CO_2 is a competitive inhibitor for ethylene action and high levels are documented to synergistically control, with low O_2, ripening in apples (Gorny and Kader 1996). Elevated CO_2 can also inhibit the growth of spoilage fungi but this effect is attributed to the effect of the gas on the interaction between the pathogen and the fruit tissue, with high CO_2 delaying ripening through competitive inhibition of ethylene action and hence delaying onset of susceptibility of the tissue to infection (Burton 1978). High CO_2 levels also inhibit the Kreb's cycle of oxidative metabolism through a mechanism of substrate accumulation and feedback inhibition (Burton 1978; Fidler and North 1967b). The long term result could be accumulations or alternate metabolic utilization of respiratory intermediates, such as characterized as declines in malic acid and increases of succinic acid in 'Bartlett' pears (Williams and Patterson 1964). Elevated CO_2 levels, used in storage, have recently been found to influence gene expression in strawberries with demonstrated linkages of gene expressional changes to postharvest physiological responses (Ponce-Valadez *et al.* 2008). How these changes in gene expression, in response to high CO_2 levels, are mediated is not known at this time, but the use of molecular techniques may help to elucidate this issue in the future.

The use of elevated CO_2 with low O_2 (controlled atmospheres) in tandem with low temperatures provides demonstrable enhancement of storage potential for apple fruit (see **Table 1** for comparison of air versus CA storage potentials of different apple cultivars). Thus, it can be concluded that application of combined treatments provided enhanced quality retention in apples. In **Table 1**, it should be noted that some apple cultivars, such as 'Delicious' are tolerant to ultra low oxygen and can benefit with O_2 storage levels significantly below 1%. Ultra-low oxygen provides a significant benefit beyond that achieved with conventional low O_2 CA storage, in that it can provide a good control for superficial scald (Lau 1990). Low ethylene was another enhancement to CA storage that was investigated for control of scald, however, the consistency of that approach was limited by the uniformity in maturity of apples going into low ethylene storage since it is difficult to control ethylene accumulation in rooms with mixed maturity fruit (Lau 1999). Another drawback for low ethylene storage is the cost of the equipment required to remove ethylene from the storage atmosphere (Lau 1999). However, a very recent addition to the CA storage protocol has been the application of the ethylene inhibitor, 1-methylcyclopropene (1-MCP). It is applied in conventional low temperature plus CA storage of apples to further enhance quality retention and extend storage and shelf life of many commercially-grown apple cultivars (Watkins and Ekman 2005). The previously anticipated benefits of low ethylene atmospheres are now instead achieved by blocking ethylene action with 1-MCP, providing excellent quality retention and control of scald (Watkins and Ekman 2005).

Table 1 Relative maximal storage potential of six commercially-grown apple cultivars when stored either in conventional low temperature storage or optimally developed controlled atmosphere conditions.

Cultivar	Maximum Storage Potential (Months)	
	Air Storage at 0 ºC	Optimal Controlled Atmosphere[1]
'Delicious'	3	12 (0.7-2 kPa O_2 + 2 kPa CO_2)
'Gala'	2-3	5-6 (1-2 kPa O_2 + 2-3 kPa CO_2)
'Golden Delicious'	3-4	8-10 (1-2 kPa O_2 + 2-3 kPa CO_2)
'Granny Smith'	3-4	10-11 (1.5-2 kPa O_2 + 0.5 kPa CO_2)
'Law Rome'	3-4	7-9 (2 kPa O_2 + 2-3 kPa CO_2)
'McIntosh'	2-3	5-7 (3 kPa O_2 + 5 kPa CO_2)[2]

Note: Extracted from Watkins *et al.* 2005.
[1] the optimal atmosphere composition is provided in brackets. [2] the CO_2 is 2-3 kPa for the first month and is then raised to 5 kPa.

The challenges facing today's marketplace is that the mechanisms of the physiological bases for the success with apples in CA be fully understood so that equally effective treatments can be designed and appropriately extended to other products without preconceived restrictions on what treatments will be applied. The intent is not to encourage application of controlled atmosphere storage for all produce, since the benefits to many other fruits and vegetables do no necessarily justify this (Kader 1980). However, the target effects on quality in the product that need control must be dissected out and appropriate treatment combinations developed to provide the best integrated control of quality decline for each specific case.

OTHER COMBINATION TREATMENTS FOR FRUITS AND VEGETABLES

One of the areas receiving greater attention, in regard to combined treatments, is in the development of "greener" postharvest practices (Ben-Yehoshua 2005). There are a multitude of environmental and social reasons to find alternatives to conventional chemical fungicides. Therefore the use of common food additives in combination with low, but non-chilling, temperature (7.2°C) and CA (5 kPa O_2 +15 kPa CO_2) to improve the storage and shelf life of products such as pomegranates is becoming more attractive for that commercial industry (Palou *et al.* 2007). Since pomegranates are chilling sensitive, there is a need to store them at elevated temperatures and to then apply the anti-fungal treatment, in addition to CA, to control the growth of *Botrytis cinerea* (gray mould). The elevated temperature storage avoided the risks of chilling-induced quality defects on the pomegranate fruit. Because pomegranate is not climacteric in its ripening pattern, the elevated temperatures are less problematic to quality control, but water loss is considered the main limitation to its storage life (Palou *et al.* 2007).

Using a similar approach, Usall *et al.* (2008) found that the use of a biocontrol agent, *Pantoea agglomerans* CPA-2, in combination a sodium bicarbonate dip could be effective to control *Penicillium digitatum* in 'Lanelate', 'Eureka' and 'Valencia' oranges stored at 10°C. Again the fruit are stored at the warmer temperature to avoid chilling-induced quality defects, but this temperature enables the growth of the *P. digitatum*. Like with pomegranate, oranges do not have a climacteric ripening pattern and so water loss and decay are the most important factors limiting storage life.

The control of blue mould (*Penicillium expansum*) in pome fruit has been significantly improved with the application of combined treatments, while storing at low temperatures. In the first instance, application of a short warm water (46°C) treatment, followed by the surface inoculation with a yeast antagonist (*Rhodotorula glutinis*) has been shown to synergistically control blue mould in Nashi pears (Zhang *et al*. 2008). In that example, fruit were held in cold air storage conditions. In a second example, it was found that two yeast antagonists (*Metschinkowia pulcherrima* and *Cryptococcus laurentii*) in addition to a 2% (w/v) dip in sodium carbonate and with the use of commercial controlled atmospheres gave synergistic control for blue mould on 'Golden Delicious' apples (Conway *et al*. 2007). Each of the treatments provided some control, but when all four were applied in series, then the complete control was possible under laboratory conditions. Those workers took the next step and tested the combined treatment protocol in commercial conditions and confirmed that it was an effective and practical approach in industry practice (Janisiewicz *et al*. 2008). In both of the above cases, the ripening, softening and decay of the fruit was inhibited wholly or partially by controlled atmospheres and/or low temperature storage. In the case with the Nashi pears, hot water helped also control firmness loss and decay and the yeast antagonist provided added control for decay. In the second case, the sodium bicarbonate and yeast antagonists both provided synergistic inhibition of blue mould spore germination and growth on the apples.

There are two examples of the use of the ripening inhibitor, 1-methylcyclopropene (1-MCP), as a pre- or post-storage treatment for improving the storage or shelf life of pears and apples, respectively. Spotts *et al*. (2007) found the pre-storage sequential treatment with 1-MCP in conjunction with gaseous hexanal provided a synergistic control of several decay-causing organisms in 'd'Anjou' pears stored in semi-commercial conditions. Lu and Toivonen (2003) found that a simultaneous co-application of 35 kPa CO_2 and 1-MCP for 16 h resulted in a synergistic enhancement of firmness and quality retention of 'Gala' apples in simulated shelf conditions (20°C). The fruit treated with the combined treatments showed delayed onset of ripening and better titratable acidity retention. However, the post-storage effectiveness of the treatment was limited if the 'Gala' apples had initiated climacteric ripening before treatment. These two examples show that the application of combined treatments at a point in time of handling of fruit can have residual activity in subsequent storage or distribution quality retention of those fruit.

Lemoine *et al.* (2008) reported that combined treatments can be used to maintain quality of a highly perishable vegetable such as broccoli at elevated storage temperatures with the use of a novel combined treatment. The authors found that a treatment using hot air at 48°C for 3 h and an 8 kJ m^{-2} dose of UV-C irradiation resulted in synergistic preservation of green color and protein content in treated broccoli that was held at 20°C, in the dark. This suggests that combined treatments may lead to less reliance on refrigeration for handling perishable products such as broccoli, perhaps reducing energy requirements in short-distance, local distribution chains.

There has been much research activity looking at botanical microbe and fungal inhibitors showing significant benefits in both CA and MA storage regimes (Ben-Yehoshua and Mercier 2005). However, on critical analysis it becomes clear that the potential for commercial application is limited due to the fact that these compounds need to be applied at such high relative concentrations that they lead to quality or taste defects that make the produce unappealing to the consumer (Ben-Yehoshua and Mercier 2005). This analysis highlights the importance of considering potential impacts on consumer acceptance when developing combined treatment strategies, especially when the treatment leaves a residue on the fruit or vegetable.

COMBINED TREATMENTS FOR FRESH-CUT FRUITS AND VEGETABLES

The concept of combined treatments has been explored more extensively in fresh-cut fruits and vegetables, probably because the challenges in those commodities is far greater than in whole product (Toivonen and DeEll 2002; Toivonen 2007).

In two cases, the ethylene antagonist 1-MCP has been applied as part of a combined treatment (Aguoayo *et al.* 2006; Kim *et al*. 2007). The combination of 1-MCP (applied before or after processing), a 1% (v/w) $CaCl_2$ dip and a modified atmosphere (3 kPa O_2 + 10 kPa CO_2) resulted in a synergistic enhancement in shelf life of fresh sliced strawberries (Aguayo *et al*. 2006). Shelf life at 5°C increased from 6 to 9 days with the combined treatment. It could be inferred that 1-MCP antagonized ethylene action in the sliced strawberries which would have otherwise led to premature softening of the sliced strawberries. The $CaCl_2$ would have been expected to increase the firmness and cell integrity of the sliced strawberry tissue (Toivonen and Brummell 2008). The high CO_2 is expected to have provided significant control of microbial growth (see discussion on mechanism of high CO_2 effects earlier in the review). Kim *et al*. (2007) found that a pre-treatment followed by washing with acidified $NaClO_2$ reduced microbial growth, decay and deterioration in fresh-cut cilantro. It is likely that the role of 1-MCP was to control ethylene effects on cilantro yellowing and tissue break down and that the acidified $NaClO_2$ controlled microbial growth. In both examples, the 1-MCP application was associated with control of ethylene-induced effects that would have led to tissue break down and softening. The other treatments applied either supported this effect in a synergistic manner (i.e. $CaCl_2$) or targeted control of microbial growth (i.e. high CO_2 or acidified $NaClO_2$).

Two studies on combined treatments in fresh-cut tropical fruits have concentrated on treatments involving optimization of modified atmospheres for those products. Marrero and Kader (2006) determined that fresh-cut pineapple was best held at 5°C or colder and at O_2 concentrations of 8 kPa or lower and CO_2 at 10 kPa. The temperature was essential to control microbial growth, lowered O_2 was important to keeping the yellow color of the pulp and the high CO_2 to reduce the browning. In another study, de Souza *et al.* (2006) found that a combined treatment of a post-cutting application of 3% (w/v) $CaCl_2$ and a low O_2 atmosphere (2.5 kPa) resulted in a synergistic improvement in shelf life for fresh-cut mango held at 3°C. In that study, CO_2 levels were found to have little influence on quality retention. These two reports emphasize the need for fresh evaluation of the benefits specific combinations of elevated CO_2 and low O_2 in fresh-cut fruits, even though there are established guidelines for whole fruit.

Warm water treatments have been cited as components of combined treatment protocols in whole fruit and vegetable products, but there are also specific benefits for their use in fresh-cut products. In the case of fresh-cut iceberg lettuce, the need to consider irradiation to control food-borne human pathogens has raised the need to develop combined treatments in order to avoid irradiation-induced quality loss in that commodity (Fan *et al*. 2003). A dip in 47°C water for 2 min followed by packaging in modified atmosphere packages and exposure to 0.5 and 1 kGy γ-irradiation resulted in the best overall quality retention and lowest cut-edge browning. This approach may make the use of irradiation feasible for fresh-cut lettuce, a vegetable which is very sensitive to irradiation treatment. Aguayo *et al*. (2008) found that application of hot water dips (60°C) containing calcium salts resulted in significant decreases in softening and microbial growth in fresh-cut 'Amarillo' melon. The calcium salt used was important, calcium lactate and chloride in the hot water resulted in a 2 log reduction in total plate counts, whereas calcium propionate in the water resulted in a 4 log reduction. However, they also found that the 0.5% concentration of calcium propionate produced and off-flavor in the melon pieces (Aguayo *et al*. 2008). These two papers provide good insight into the benefits of using warm or hot water treatments as a platform in a combination treatment protocol for fresh-cut fruits and vegetables. However, a there must be a caution raised as there is evidence to indicate that warm or hot water treatments can lead to increased risk in food-borne pathogen growth on fresh-cut products (Delaquis *et al*. 2002).

Finally, a more controversial treatment, super-atmospheric O_2 (Kader and Ben-Yehoshua 2000) has been presented as part of a combined treatment protocol for fresh-cut vegetables (Escalona *et al*. 2006; Conesa *et al*. 2007). While fresh-cut lettuce is generally packaged in 1-8 kPa O_2 and 10-20 kPa CO_2 to preserve quality (i.e. preventing browning and microbial growth), this often leads to metabolic disorders that give off-flavours (Jacxsens *et al*. 2002). A combination of at least 80 kPa O_2 with 10-20 kPa CO_2 provided a treatment that maintained visual quality in butterhead lettuce, while avoiding metabolically-induced off-flavours (Escalona *et al*. 2006). A similar approach of using a combination of 50 or 80 kPa O_2 with 15 kPa CO_2 resulted in a significant improvement in sensory quality and reduction of microbial growth of fresh-cut peppers stored at 5°C after cutting (Conesa *et al.* 2007). Both of these papers show that co-application of high CO_2 is an important feature of the effects associated with high O_2 and so this must be considered when developing treatment protocols involving high O_2 (Kader and Ben-Yehoshua 2000).

CHILLING INJURY CONTROL IN SENSITIVE FRUITS AND VEGETABLES

Chilling injury is a broad-based problem for many sub-tropical, tropical and temperate fruits and several 'fruit-vegetables' such as tomato, cucumber and zucchini squash (Morris and Brady 2005). It is probably the most pervasive problem facing the marketing of these commodities since the general principle for postharvest handling requires the application of low temperatures to delay deterioration and control decay (Morris and Brady 2005). There have been numerous approaches proposed to alleviate chilling injury, including: 1. intermittent warming (Lill *et al*. 1989), 2. heat treatment (Lurie and Klein 1991), 3. applications of methyl jasmonate (González-Aguilar *et al*. 2001), 4. temperature pre-conditioning (Wang *et al*. 1992), and 5. modified atmospheres (Lill *et al*. 1989; Marrero and Kader 2006). However, none of these approaches has been shown to be sufficiently effective to completely alleviate chilling-associated quality loss or injury for most commodities.

There have been, however, improvements in the control of chilling injury with the use of combination of the above-identified treatments. Wang (1994) showed that the application of heat treatment and temperature preconditioning could reduce chilling more effectively than the application of either treatment alone. The basis of injury control was determined to be modulation in oxidative stress resistance in the tissues (Wang 1994). The knowledge base already developed in the area of environmental stress tolerance induction can help to explain Wang's results. It is generally known that an application of a non-injurious single stress treatment will elicit a few or several discrete physiological changes in plant tissues consequently producing a limited improvement in cross-tolerance to other stresses in the plant tissue's environment (Toivonen 2003). However, if two or more non-injurious stress treatments are applied, a wider range of responses are elicited and thus a broader stress cross-tolerance will develop in the plant tissue (Toivonen 2003). This understanding forms the theoretical basis for the use of multiple or combined treatments and their enhanced benefits to control quality-associated problems have been documented in several instances (Toivonen 2003). The molecular basis for these responses has been relatively well elucidated (Mittler 2002), and this knowledge base may potentially provide a more comprehensive approach to predicting whether treatment combinations will produce additive or synergistic outcomes in terms of stress tolerance.

Despite efforts and decades of research, there appears to not be a good solution to prevention of chilling injury for most sensitive products. At this time, there is hope, in the long term, that genetic engineering or directed breeding may provide solutions (Iba 2002; González-Agüero *et al*. 2008). Coupled with the chilling injury issue is an emerging concern for the need to consider energy use reductions in handling and transportation of fresh fruits and vegetables (Ben-Yehoshua 2005). Refrigeration is a significant component of the conventional handling of fruits and vegetables (Mitchell 1992). A critical analysis of the original principles that resulted in adoption of current handling processes and technologies will provide criteria that will allow alternate approaches for handling fresh fruit and vegetable products, particularly those that are chilling sensitive. An approach which has less reliance on conventional low temperature refrigerated storage may be considered too radical at this time. However, microbial growth could be controlled adequately with an alternative treatment approach, then low temperature may not be required to extend storage life at least for short term storage (i.e. for durations in the range of several weeks to a month). Currently, there are several fruits that are stored at warmer temperatures. A good example is greenhouse-grown tomatoes are commercially stored and handled at higher temperatures (13°C or above) to preserve their flavour quality which is impaired by low temperatures (Maul *et al*. 2000). The reluctance to hold many other products at warmer temperatures may relate to practical considerations that pertain to transport across long distances from production to market, rapid ripening rates at warmer temperatures and risk of microbial growth and decay.

With the advent of 1-methylcyclopropene (1-MCP), the ability to at least partially control climacteric ripening and softening without lowering temperature has become feasible (Toivonen and Lu 2006). However, associated with warmer temperatures is the increased potential for postharvest decay (Sommer 1992). Hence, in order for warmer temperature handling to become feasible, co-treatments which inhibit microbial growth and decay are required. Initial work with 'Sunrise' apple, a summer apple which is chilling-sensitive, has shown that storage at elevated temperatures of 15°C with the application of 100 nL L^{-1} 1-MCP can provide one month of good quality retention without significant decay issues (Toivonen and Lu 2005). The four week storage potential would certainly be considered reasonable for marketing a low production volume summer apple such as 'Sunrise'. In the case of greenhouse tomatoes, the application of 15 nL L^{-1} 1-MCP can provide three weeks of good quality retention when treated and stored at 15°C (Mostofi *et al*. 2003). However, it must be stated in the case of this tomato study, that a commercial fungicide was applied prior to 1-MCP treatment on the tomatoes. Hence these results must be considered within the context that good fungal decay control was achieved by using a postharvest fungicide dip. Elevated temperature storage with 1-MCP to control ripening may also confer a nutritional benefit since it has been shown that bioaccessible content of phenolic antioxidants we significantly higher in 1-MCP-treated fruit stored at temperatures of 15°C or higher than in treated and untreated fruit stored below 15°C (Qiu *et al*. 2009). 'Sunrise' apples not treated and stored above 15°C had lower bioaccessible contents of phenolic antioxidants than those stored at cooler temperatures. It has been also shown that storage of chilling-sensitive tomatoes at temperatures greater than 12.5°C results in improved sensory (flavour) quality

Table 2 The efficacy of a co-release technology sachet (NT) in controlling softening and decay in 'Harrow Beauty' peaches after four weeks storage at 15°C in modified atmosphere packages (MAP) having steady-state atmospheres of 4 kPa O_2 + 15 kPa CO_2 (Toivonen and Lu, unpublished data). Control treatment consisted of MAP without the addition of the NT sachet. The NT sachet consisted of a mixture consisting of (by weight) 13% sucrose, 9% SmartFresh® powder, 26% dried brewers yeast, 52% sorbitol (Toivonen and Lu 2006). The resultant 1-MCP concentration in the headspace was measured as 1 μL L^{-1}.

Treatment	Firmness (N)[1]	Decay rating (1-5 scale)[2]	Fruit with decay (%)
Control + MAP	17.2 ± 1.2	3.6 ± 0.9	88.9 ± 11.1
NT + MAP	37.3 ± 3.9	1.0 ± 0.0	0.0 ± 0.0
Significance level	<0.0001	0.0456	0.0013

[1] Crisosto and Kader (2005) state that peaches in the firmness range of < 27 to 36 N measured on the fruit cheek have high consumer acceptance

[2] The rating scale was: 1 = no decay, 2 = slight decay visible, 3 = moderate decay visible, 4 = severe decay visible, and 5 = fruit completely decayed. (Toivonen and Lu, unpublished data)

(Maul *et al*. 2000). Hence, there may be many direct benefits to the consumer with the use of an elevated temperature approach to handling chilling-sensitive, climacteric fruit and application of combined treatments.

A recent patent application has described the concept of a "co-release" technology which releases approximately 1 μL L^{-1} 1-MCP to control ripening while enabling, in parallel, the accumulation of biologically significant levels of ethanol, acetaldehyde and CO_2 that have been demonstrated to inhibit decay organisms at elevated storage temperatures (Toivonen and Lu 2006, 2007). There are two scenarios where this concept may provide new opportunities to the fruit and vegetable industry; 1) the handling of chilling sensitive, climacteric fruits, particularly tropical fruit, at non-chilling temperatures, and 2) the development of fresh-cut mixes containing climacteric fruit and ethylene-sensitive fruits or vegetables (Toivonen and Lu 2007).

Peaches are an example of a climacteric fruit which susceptible to chilling injury in common storage temperature ranges (Lill *et al*. 1989). They are also very susceptible to posharvest decay during storage and transport (Lill *et al*. 1989). Commercial handling of peaches has involved making compromises, the most common of which is to store the fruit at 0°C or slightly higher since the symptoms progress very slowly at that temperature range (Mitchell *et al*. 1974; Lill *et al*. 1989). Some cultivars are more susceptible than others (Lill *et al*. 1989) and so other approaches to control the problem have been developed, with varying degrees of success. However, flavour of peaches subjected to low temperature storage has not been critically evaluated. It may be that peaches, when stored at chilling temperatures, suffer similarly as do tomatoes in regards to flavour loss despite the fact that chilling injury has not become visibly apparent (Maul *et al*. 2000). A combined treatment approach has been demonstrated that will control ripening (softening) and decay of peaches when held at 15°C, which is above chilling threshold for peaches (**Table 2**; Toivonen and Lu 2005). The results show promise since in that test, decay at four weeks of storage was kept to zero in the combined treatment and firmness was in a range considered to be 'acceptable to the consumer' (Crisosto and Kader 2005). Since the goal of handling soft fruits such as peaches is to ensure time to get the product to the market and consumer, the time afforded by such handling and elevated storage temperatures could be considered for commercial application. Further evaluation of this type of approach is warranted and commercial implementation will likely result in delivery of better fruit taste quality and functional value to the consumer.

IDENTIFYING SYNERGISTIC COMBINED TREATMENTS

Many of the studies discussed in this review have relied on the use of analysis approaches providing limited information on response to individual and combined treatments, for example visual assessment of a disorder severity or measurement of enzyme activity changes. In addition, larger experimental units are required to gain the sensitivity to clearly quantify treatment responses. The reality has been that improvements and development of successful combined treatments has required a significant effort to run large numbers of experiments to assess the ultimate effects on quality, shelf life and safety of the fresh fruit and vegetable product.

In today's research climate, there is likely little support for such large experiments particularly since they do not advance basic mechanistic science and industry generally does not have the resources conduct the work, at an applied level, on a meaningful scale. Increased application of molecular approaches (genomics, proteomics and metabolomics) can potentially provide relatively rapid answers to the questions raised in this review (Renaut *et al*. 2006; Pedreschi *et al*. 2008), provide problems that will be attractive to young scientists searching for training opportunities and expedite the development of solutions for the long term for fresh produce handling. The "omic" tools can provide detailed insight into genetic variability to responses to treatments (Iba 2002; González-Agüero *et al*. 2008; Ponce-Valadez *et al*. 2008), into differential responses of various treatments (Mittler 2002; Gray and Heath 2005; Kaplan *et al*. 2007), and integrated effects of multiple treatment combinations (Mittler 2002; Pedreschi *et al*. 2008). Already, there has been some progress in understanding the complexity of gene expression differentials in chilling sensitive and resistant peaches which underline the value of gene expression studies on elucidating the mechanisms or physiological processes leading to storage disorder development (González-Agüero *et al*. 2008). Since the gene expression, protein expression and metabolite accumulations response to treatments can be quite broad and diverse in nature (Mittler 2002), it is essential to develop evaluation tools that allow capture of this diversity. Molecular approaches provide the tools that meet these criteria. These tools are relatively well developed as demonstrated by the papers cited previously in this paragraph and there also is much information available to be applied in postharvest systems from the botanical stress tolerance literature (Mittler 2002).

CONCLUSIONS

Application of novel combinations of treatments to control quality decline in fruits and vegetables is an area that deserves greater effort, particularly since there is a move towards more environmentally friendly technologies that do not use chemical controls for decay (Ben-Yehoshua 2005). The success of such an approach will be to identify complementary or synergistic co-treatments to gain a commercially desirable quality and safety for the fruit or vegetable product. Hence effort must be placed into understanding the mode of action of postharvest treatments at the genomic, proteomic and metabolomic levels. Such understanding will allow improved and accelerated selection of co-treatments having complementary or synergistic effects. The implementation of more radical approaches that may use elevated storage temperatures for chilling sensitive whole fruits will be ultimately dependent on whether data will support breaking away from existing commercial practice while also satisfying concerns of regulatory agencies over implications on consumer health and safety.

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