

Is it possible to find an optimal controlled atmosphere?

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Abstract

Every controlled atmosphere (CA) conference has included recommendations for controlled atmospheres that purport to produce the optimal storage atmosphere for specific horticultural crops. However, the natural variability in the raw material and its dynamic response to processing and storage conditions may render it impossible to identify a truly optimal storage atmosphere. Additional refinements in recommendations for the CA and modified atmosphere (MA) storage of fruits and vegetables will continue to accrue through empirical observations derived from traditional experiments in which the six components of the storage environment (i.e. duration, temperature, relative humidity, O₂, CO₂ and ethylene levels) are varied in well-defined steps. However, the variability inherent in biological systems and the often dynamic response of the stored commodities to changes in their storage environment may not be adequately accounted for in these types of static experiments where all variables are usually held constant for the duration of the experiment. Truly significant advances in the use of CA and MA may require the development of mathematical models that incorporate some measure of the commodity's dynamic response to the storage environment. These measurements should reflect the commodity's changing response to various storage parameters (e.g. a shifting anaerobic compensation point), and should be useful in predicting future changes in quality.

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1. Introduction

Recommendations for the controlled atmosphere (CA) and modified atmosphere (MA) storage of fruits and vegetables have been included in the proceedings of the International CA and MA Conferences. Since the start of this series of

conferences in 1969, the recommendations have been periodically revised because of additional information and technological innovations and expanded to include new species and cultivars. Specific lists of CA and MA recommendations were included in the 1977 meeting (Lipton, 1977; Morris and Kader, 1977; Weichmann, 1977), and every meeting since 1985 (Saltveit, 1985, 1989, 1993, 1997a, 2002). These recommendations give the temperature, ranges of O₂ and CO₂, and potential beneficial and harmful effects for the CA and MA storage of horticultural crops.

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As the years have passed, the recommendations have become more refined and narrowed (e.g. to individual cultivars, growing locations, and handling procedures). Changes to the recommendations are now only made when a preponderance of new data suggest a re-evaluation is warranted. The advent of fresh-cut and MAP have stimulated research in finding the optimal storage atmosphere for even more narrowly defined products and storage conditions. After having compiled recommendations for the CA storage of vegetables for the past five conferences (Saltveit, 1985, 1989, 1993, 1997a, 2002), I have come to question from where further substantial refinements will arise (Saltveit, 1997b). The natural variability in the raw material and its response to processing and storage conditions may render it impossible to identify a truly optimal storage atmosphere with our current techniques and methodologies.

The vast majority of research on the CA storage of vegetables continues to be studies of modified atmosphere packing (MAP). The procedure most often used is to enclose a small amount of commodity in a film package, and periodically monitor the resulting atmosphere and product quality. If all variables are controlled, the internal atmosphere equilibrates at the recommended and projected levels. However, this approach has a number of limitations in selecting an optimum MA and in furnishing useful information on respiration rates and optimum CA composition. A major problem is that the consumption and production of O₂ and CO₂ by the commodities are metabolically interrelated, making it difficult to study the effect of one gas independent of the other. The uncontrolled nature of the atmosphere in the package and the length of time required to reach equilibrium further complicate interpretation of the results. Even such minor aspects as product orientation, package orientation, and location of perforations can significantly alter the equilibrium gas concentration (Ngadi et al., 1997). New methods of using MAP promise to overcome these limitations and furnish useful information on respiration rates and optimum atmosphere (Aki-moto and Maezawa, 1997; Banks and Nicholson, 2000; Beaudry, 2000; Chen et al., 2000; DeWild and Peppelenbos, 2001; Xu and Yu, 2000).

2. What variables can be controlled?

The six primary environmental variables usually controlled in CA, MA and MAP are storage duration, temperature, relative humidity, and the concentrations of O₂, CO₂, and ethylene. The ‘optimum’ storage environment for each commodity is designed to maintain these variables within a set of limits that produces the maximum storage life for most of the individual members of the commodity. These storage variables could be thought of as a volume in six-dimensions. Each side of the resultant polyhedron would be a plane subscribed by two of the variables. By holding four of the variables constant (usually duration of storage, temperature, relative humidity, and ethylene concentration), the space can be collapsed to a more easily envisaged two-dimensionally planar graph that shows the recommended concentrations of O₂ and CO₂ for optimal storage (Fig. 1). However, we should remember that any significant change in the remaining four variables could generate another set of two-dimensional graphs.

The space delineated for the optimal CA for one commodity (e.g. iceberg lettuce or mature-green tomatoes) contains within its boundaries a number of smaller spaces representing the ‘optimal’ storage conditions for ever more homogenous groupings of the commodity (Fig. 2). The importance of defining storage conditions for small homogenous groups within the broader commodity recommendations can clearly be seen in the extensively studied case of apples. Fruit of the major cultivars and the major growing locations have specific recommendations that often differ significantly from one another. Yet even within these refined storage conditions, fruit from different growing seasons and parts of the tree, and those fruit experiencing different cultural practices and harvesting techniques can respond differently to the ‘optimum’ storage environment.

Other environmental variability that influences the response of the commodity during storage include microbial load, light, orientation of the product in the gravitational field, and the concentration of other gases; the most important being ethylene. Ethylene is added to stimulate the ripening of certain fruit, but its general effects

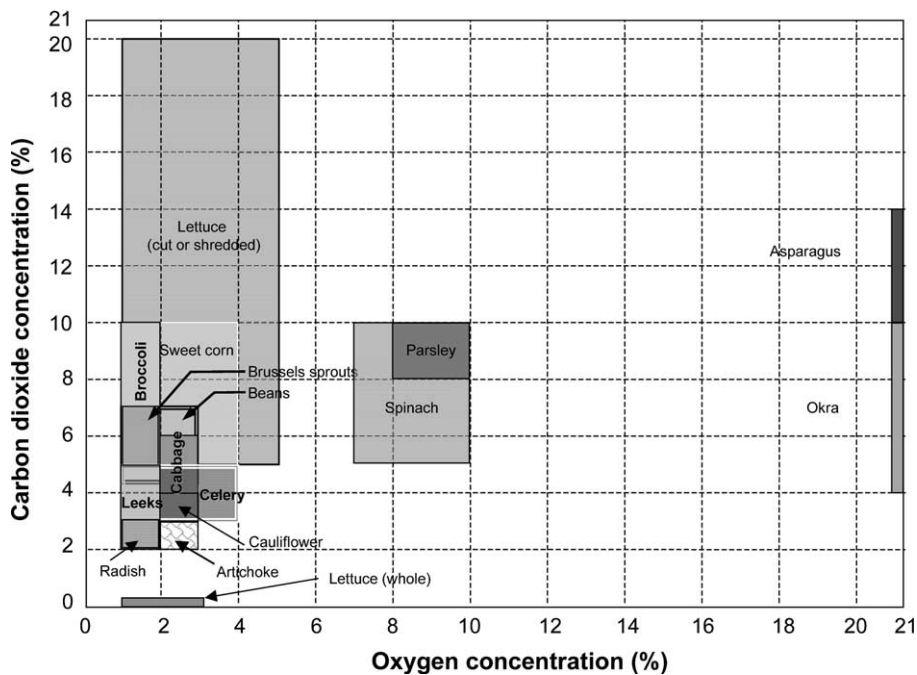


Fig. 1. Recommended oxygen and carbon dioxide ranges for the storage of some harvested vegetable commodities (Saltveit, 2002).

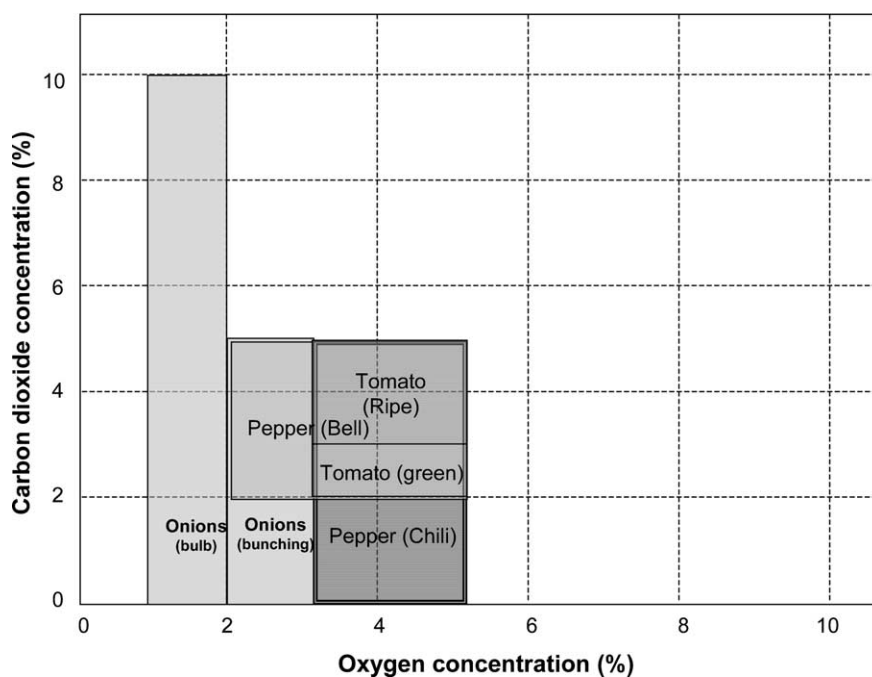


Fig. 2. Recommended oxygen and carbon dioxide ranges for the a few harvested vegetable commodities showing differences within individual commodities (Saltveit, 2002).

are usually detrimental to storage life and quality (Saltveit, 1999). Therefore its concentration or activity should be minimized to maximize product storage life.

3. Problems with finding an optimal CA

3.1. Static not dynamic measurements

Currently, an optimal CA is found by taking selected ‘snap-shots’ of commodity specific quality parameters under a specific combination of storage conditions (Fig. 3). For example, some aspects of quality (e.g. color, firmness, and soluble solids) are measured after a defined period of storage under specific continuous storage conditions (e.g. temperature, relative humidity, and concentration of O₂, CO₂, and ethylene). How-

ever, the storage requirements can vary with duration of storage simply because some quality parameters change at different rates than others. Conditions that maintain optimum quality during 2 weeks of storage may be detrimental if continued for significantly longer periods of time.

3.2. Biological variability

Biological variability coupled with the development of new cultivars, storage technologies, and packaging, widens rather than narrows the ‘optimal’ range of recommended O₂ and CO₂ levels. The ranges for these gases must encompass those used in sophisticated ultra-low O₂ storages, and those used in MA packages subjected to temperature abuse. After a time these general recommendations become too diffuse and the commodity must be segregated into narrower and narrower

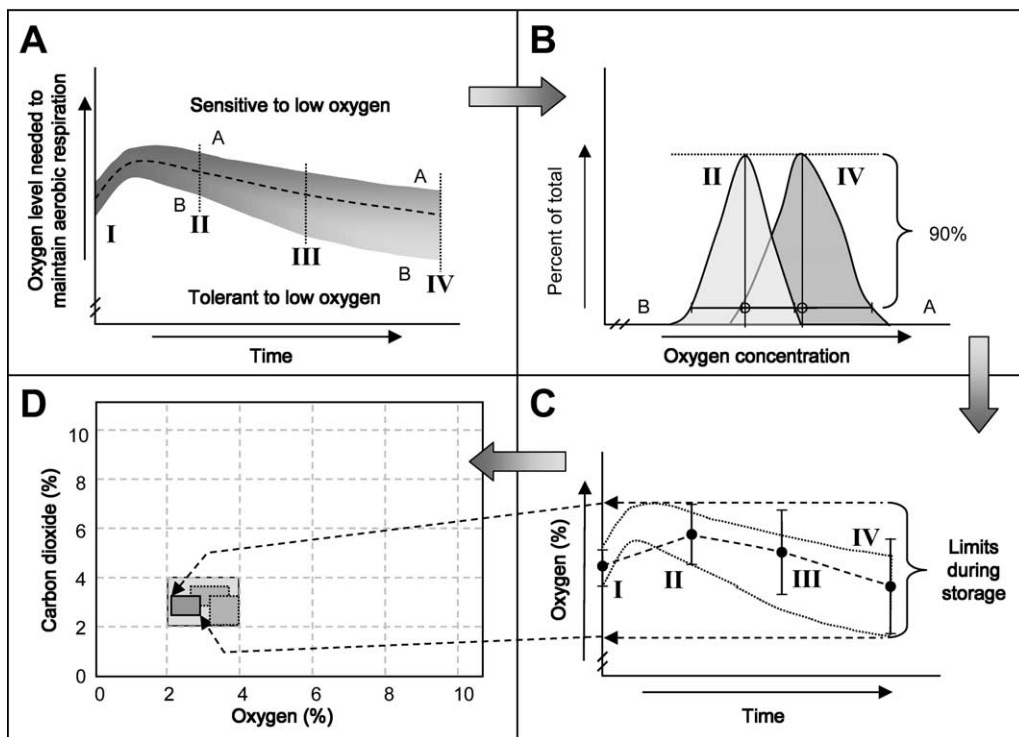


Fig. 3. Steps in finding the optimal oxygen concentration for storage of a hypothetical commodity. (A) Within one experiment, the range of oxygen concentrations needed for aerobic respiration decreases with duration of storage. (B) At two times (II, IV), the percent of individuals tolerant and sensitive to low oxygen changes as does the mean value. (C) The oxygen limits for 90% of the population shifts over time in storage. (D) The oxygen limits for storage circumscribes an area, but data from additional experiments expands the recommended range to fill an area delineated by whole numbers.

categories, each with their specific recommendations.

Other major problems include the differences within an individual commodity (e.g. epidermis, vascular, core, etc.), the differences among individual commodities (e.g. cultivar, growing conditions), the differences with age (e.g. ripening, senescence), and the differences among quality parameters (e.g. appearance, firmness, flavor, vitamin content).

3.3. *Sense and control technology*

The recommended conditions for each storage variable operate within a range governed by the precision of measurements and control measures. For example, the composition of the atmosphere (i.e. N₂, O₂, CO₂) could be measured in the $\mu\text{l l}^{-1}$ range (i.e., ppm), and the duration of storage in milliseconds, but such exactness would be superfluous given the inherent variable response within each group of commodities, the slow responses of the stored tissue to changes in the atmosphere, and the significant lag that intervenes between initiating a change in the atmosphere and sensing the change. However, refinements in the instruments used to measure the important storage gases and their automation has led to computer controlled storages that are able to maintain storage conditions as close to the recommended values as is economically warranted. With such sophisticated ability to sense and control the CA, the question arises whether the current recommendations are truly optimal over the storage life of the commodity.

3.4. *Mutually exclusive parameters*

The CA or MA used to optimize the storage life of some quality parameters may be mutually exclusive. For example, high levels of CO₂ can control mold, reduce ethylene effects, and reduce chlorophyll loss. However, the same elevated levels of CO₂ can also promote anaerobic respiration and phenolic metabolism in some commodities. Low O₂ reduces respiration, and ethylene synthesis and action, but it also stimulates anaerobic

respiration, the production of off flavors, and possible microbe growth.

4. **What would be an optimal storage atmosphere?**

An optimal storage environment could be defined as those storage conditions that produce the best quality product. The first problem encountered is the meaning of quality. Quality can be defined as some desirable physical aspect of the commodity (e.g. weight, shape, color, firmness, aroma, sugar content, etc.) that can be measured either subjectively or objectively. Combinations of attributes and their relative weight and interactions further complicate the formulation of quality criteria. As is obvious from this definition, the exact measures that will be used to differentiate levels of quality will vary with the commodity, its intended use, and the preferences of the consumer. Quality criteria for tomatoes differ from lettuces. Criteria for pickling cucumbers differ from those for slicers. Some people prefer bananas with green tips, while others prefer more mature fruit with brown spots.

The introduction of new commodities, new cultivars, and new market segments (e.g. working single parents) makes it almost impossible to formulate strict universal descriptions of quality. However, in general the commodity should be free of physical defects, and not diseased. But even these criteria may not be universal, since some people may see slight evidence of defects or disease as proof that the product had been grown organically and free of chemical pesticides. While others may equate the surface defect of russetting of apples with increased sweetness.

The definition of an optimal storage environment could then be modified to one that preserves optimal quality for an economically viable portion of the stored commodity. Since within the sample there is variability in response to each of the storage parameters, storage conditions could either be selected to maintain quality for most of the commodity (e.g. 95%), or to limit damage to the most sensitive 10% of the population. For example, respiration would be significantly reduced and storability increased for all heads of

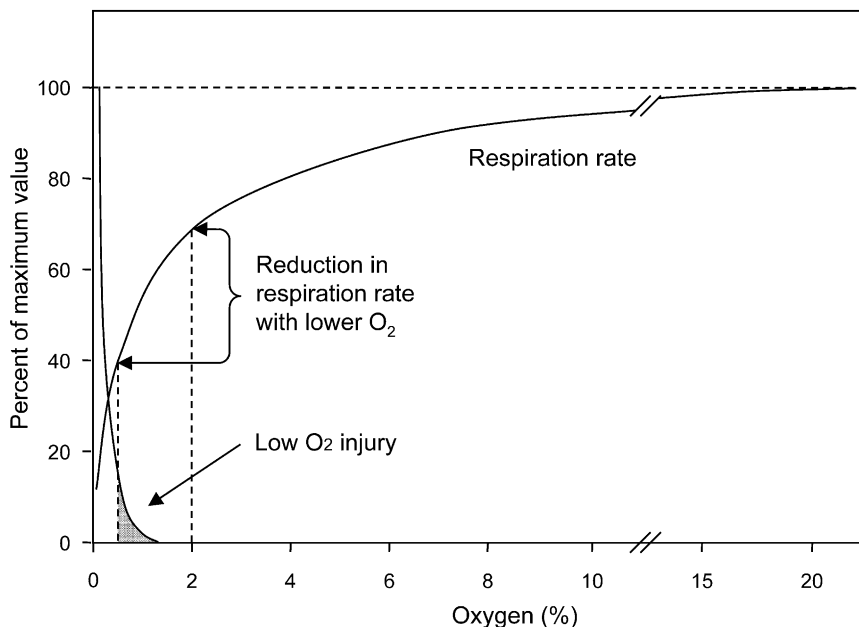


Fig. 4. Respiration of lettuce in varying oxygen atmospheres. Respiration is reduced when the oxygen concentration is reduced from 2 to 0.5%, but there is a concomitant increase in the percentage of heads damaged by low oxygen injury.

lettuce stored in a 2% O_2 atmosphere (Fig. 4). While some heads of lettuce would be lost due to disease, desiccation or damage, almost none would be lost as a direct effect of their response to the low O_2 storage atmosphere. In contrast, a 0.5% O_2 atmosphere would further reduce respiration and extend storability, but at the expense of inducing anaerobic respiration in say 10% of the stored heads. In the first case the atmosphere is selected because it produces the best results for the entire population stored. The second case shifts the focus from the majority of the group to the most sensitive individuals; or to some portion whose loss could be tolerated if it maximized the quality and storability of the remaining individuals.

5. A possible new paradigm

This new concept for an optimal atmosphere shifts our attention from the majority of individuals to those most sensitive to the storage conditions. Research under this new paradigm would focus on finding methods to narrow the range of variation, or increase the tolerance of the

most sensitive individuals. Treatments to minimize variation among the stored sample include the traditional ones of selecting cultivars with uniform responses, and groups that are more uniform in size, shape, or maturity. Newer methods include the use of 1-MCP to reduce ethylene sensitivity, lower O_2 levels, heat-treatments (heat-shock) to reduce wound responses and chilling injury, and exotic gases (e.g. CO, NO, Ar).

6. Areas of promising research

6.1. Inhibitors of ethylene action

Low levels of ethylene promote the ripening of climacteric fruit and vegetables, but even small amounts of ethylene (e.g. $0.02 \mu\text{l l}^{-1}$) can adversely affect the storage life of non-climacteric vegetables (Wills and Kim, 1996). Ethylene can be removed from the atmosphere, or the tissue can be made less sensitive to ethylene. Ventilation is usually impractical in CA or MA. One of the beneficial effects of traditional CA is that low O_2

reduces ethylene synthesis, and low O₂ and elevated CO₂ reduces sensitivity to ethylene.

There has been a remarkable increase in the number of papers published on the use of inhibitors of ethylene action over the past decade. Carbon dioxide was the first compound recognized to inhibit ethylene action (Abeles et al., 1992; Burg and Burg, 1967; Saltveit et al., 1998). Later Beyer (1976) showed that silver ions were effective inhibitors of ethylene action. However, silver's toxicity precludes its use on stored food crops, and limits its use to ornamentals and as a laboratory tool. A number of volatile inhibitors of ethylene action (e.g. 1-MCP, NBD, PPOH) have been discovered in the past decade (Philosoph-Hadas et al., 1999; Sisler and Serek, 1997, 1999; Yamamoto et al., 1992). Pre-storage treatment with 1-MCP can render the commodity insensitive to ethylene (Hall et al., 2000; Mullins et al., 2000; Sisler et al., 1999).

Inhibiting ethylene action can have tremendous commercial benefits for the storage of sensitive commodities. However, not all ripening changes are controlled by ethylene (Guis et al., 1997) and some ethylene responses may retard disease development (Lelievre et al., 2000). Additionally, ethylene production by some non-climacteric commodities (e.g. citrus, vegetative tissue) is under negative feed-back control, so that reducing perception of ethylene will actually stimulate its production. Also, the storage life of many ethylene sensitive commodities is not terminated by their response to ethylene, but by water loss, mechanical injury, or disease development. Use of compounds like 1-MCP will not compensate for poor temperature control, handling practices, or sanitation.

6.2. Ultra-low and super-atmospheric oxygen levels

Research continues on lowering O₂ Levels. Previously, O₂ levels around 2% were the lower limit for most commodities. Currently, the lower limit for many apple cultivars (Kupferman, 1997) and vegetables (Saltveit, 1997a) is 1%, and there are credible reports of successful storage at levels around 0.2% for selected vegetables (Adamicki, 1997; Hong et al., 2000). Oxygen levels around 0.5% are still above the theoretical limit for

aerobic respiration. However, respiration and diffusion barriers within the tissue and package can produce internal O₂ levels that induce fermentative metabolism within the tissue at relatively high external O₂ levels. Anaerobic metabolites could be detected by monitoring the storage atmosphere for fermentative products (e.g. ethanol) (Peiser et al., 1997). Low-O₂ injury can also be detected in packages of fresh-cut produce by an innovative ethanol biosensor (Smyth et al., 1999).

On the other hand, claims have been made that storability is enhanced at elevated oxygen levels (Barry and O'Beirne, 2000). However, recent studies have failed to show that super-atmospheric levels of O₂ extend the storability of fresh fruits and vegetables (Fig. 5) (Kader and Ben Yehoshua, 2000).

6.3. Modeling commodity response to CA

Modeling the response of the commodity mathematically would work if all significant variables were known and the tissue responded consistently. However, our knowledge of the response of the commodity to varying CA conditions is currently too limited to model the storage response of commodities without periodic feedback from the commodities themselves under storage. Factorial experiments designed to explore the many facets of the response of the commodity to changes in the

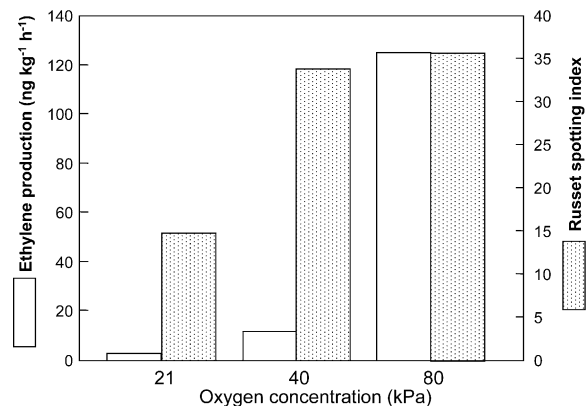


Fig. 5. Ethylene production and ethylene-induced (0.1 $\mu\text{l l}^{-1}$) russet spotting in lettuce leaf tissue held at atmospheric (21 kPa) and elevated oxygen concentrations for 10 days 5 °C (redrawn from Kader and Ben Yehoshua, 2000).

storage environment may prove to be too enormous for practical execution. A more thorough understanding of the physiology, and molecular biology underpinning the observed responses may furnish the required information to construct practical models.

6.4. Use of 'Novel' gas mixtures

Under most conditions the CA is a mix of O₂ and CO₂ with N₂ making up the balance. Expanding the repertoire of gases with unique properties for CA and MA could possibly allow modification of the physical (e.g. diffusivity) and physiological activity (e.g. inhibit ethylene perception) of the gas mixtures. Researchers have recently studied the use of CA and MA made by replacing N₂ with noble gases such as argon, and pre-storage treatment with nitric oxide (NO).

Economically interesting claims have been made for each of these mixtures (Barry and O'Beirne, 2000), but corroboration of their effectiveness and identification of possible side effects is needed before they can be recommended for a wide range of horticultural commodities. Because argon atoms are smaller and have a lower collision frequency than N₂, replacing N₂ with argon in a low O₂ CA should increase gas diffusivity and improve storability. Yet, replacing N₂ with argon or helium did not improve the storability of broccoli (Fig. 6) or lettuce (Fig. 7) in 2% O₂ CA (Jamie and Saltveit, 2002). Nitric oxide has been shown to reduce water loss from horticultural produce (Ku et al., 2000).

7. Dynamic feedback systems could control the composition of CA and MA

A more dynamic storage environment could be developed by coupling mathematical models with sophisticated sense and control instrumentation. The dynamic response of the stored commodity would be incorporated into mathematical models to determine the adjustments that need to be made to optimize the storage atmosphere. The dynamic interrelationships among a number of storage parameters have been investigated (DeWild et al.,

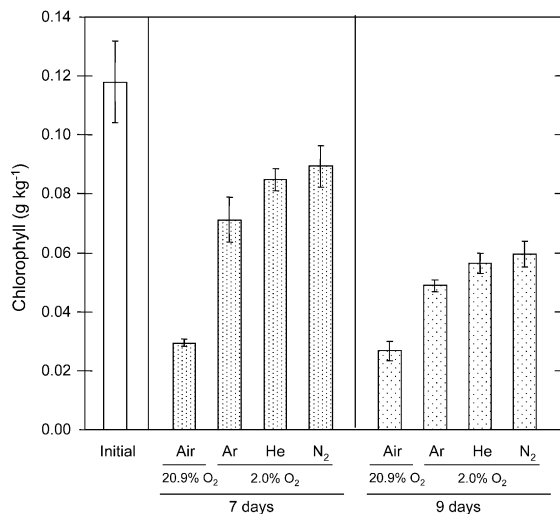


Fig. 6. Chlorophyll content of broccoli florets initially after preparation and after 7 and 9 days of storage at 15 °C in air or 2% O₂ atmospheres made with argon, helium or nitrogen (redrawn from Jamie and Saltveit, 2002).

2002; Hertog et al., 1998). Characteristics of such a dynamic commodity indicator are that it would be reliable, continuous, non-destructive, measurable at a distance from the commodity, capable of automation, and highly correlated with specific quality parameters. If research showed that a volatile product was directly related to storage life and quality preservation, then monitoring the concentration of that volatile in the storage atmosphere could be used to modify the composition of the storage atmosphere to optimize quality. However, a much better understanding of the relationships among atmospheric composition and physiological changes is needed to construct the mathematical algorithms that could project measured changes in product response into future changes in product quality.

Very little research has been done to realize the potential benefits of a dynamic feedback system. There have been no reports during the past four years on selecting a biological response that is highly correlated with quality retention and also has the consistency and predictability needed for commercial application. The lack of research is not because there are no candidates. Possible candidates include the anaerobic compensation point,

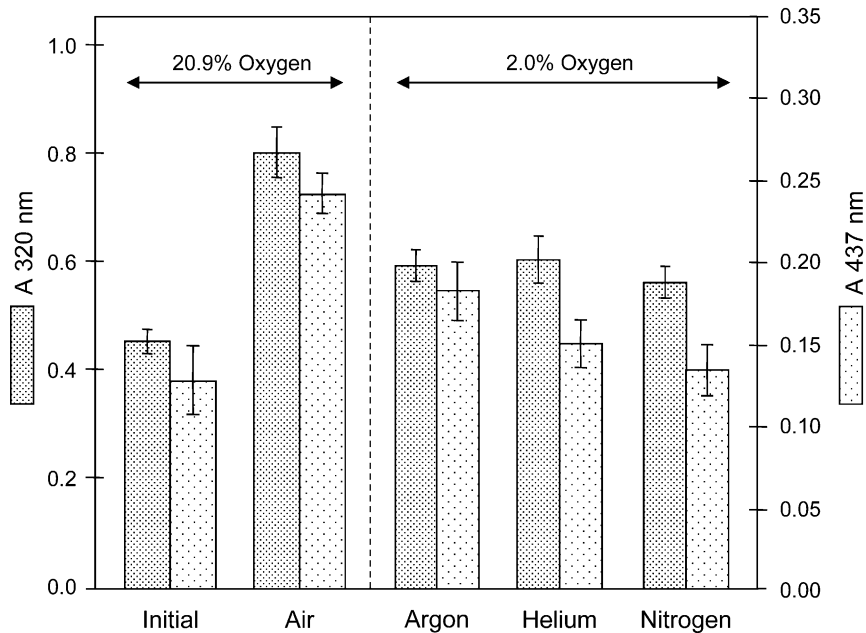


Fig. 7. Measures of the absorbance of methanol extracts of fresh-cut lettuce held for 4 days at 15 °C in air or 2% O₂ atmospheres made with argon, helium or nitrogen (redrawn from [Jamie and Saltveit, 2002](#)). Phenolic compounds detected in the extracts contribute to tissue browning.

respiratory quotient, ethylene production, specific products of anaerobic respiration (e.g. ethanol, acetaldehyde), near infrared determination of changes in composition, NMR evaluation of internal changes, external color changes, and fluorescence. Chlorophyll fluorescence has been shown to be an effective non-destructive indicator of broccoli quality in MAP ([DeEll and Toivonen, 2000](#)).

8. Conclusion

Future incremental refinements in the recommendations for the CA and MA storage of fruits and vegetables will come from identifying small homogenous groups within the larger commodity classifications that respond more uniformly during storage. Groups that respond more uniformly will permit a shift in research emphasis away from finding atmospheres that are optimal for the majority of the group to atmospheres that are detrimental to a small non-economical portion-

assuming that the loss of this portion of the commodity is compensated for by the additional quality retention for the remainder. This may not be a practical paradigm in MAP where even the slightest amount of a defective product in a package is unacceptable. Empirically derived data will continue to be collected from traditional experiments in which the six components of the storage environment (i.e. duration, temperature, humidity, and O₂, CO₂, and ethylene levels) are varied in well-defined steps. Truly significant advances in the use of CA and MA may require the use of novel storage atmospheres, and the development of mathematical models that incorporate a critical measure of the commodity's dynamic response to the storage environment. However, a much better understanding of the relationships among atmospheric composition and physiological changes is needed to construct the mathematical algorithms that could project measured changes in product response into future changes in product quality.

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