

## **Controlled Atmosphere (CA) Seminar**

### **The Biology of Controlled Atmosphere Storage**

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# The Biology of Controlled Atmosphere Storage

## Introduction

Controlled atmosphere has a place in the storage and transportation of fruit and vegetables as a supplement to refrigeration. However, controlled atmosphere storage could be described as “the icing on the cake” to good agricultural and post harvest storage. To gauge its effectiveness we need to look in detail at the biology of storage.

C.A storage is without doubt most effective on apples and pears; it is also effective on other climacteric fruit and has considerable potential on vegetables which possess high respiration rates. It is less effective on non-climacteric fruit and produce with a slow respiration rate, where quite often the only benefit is the antiseptic properties of carbon dioxide.

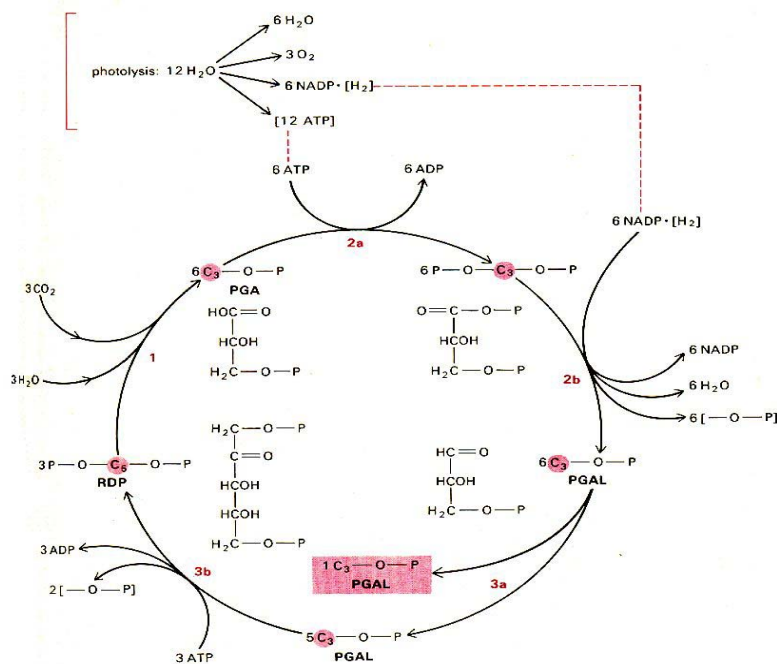
In this paper the biology of CA is examined to help illustrate where CA will be most effective within the transport of fruit and vegetables.

## Biology of Fruit and Vegetables

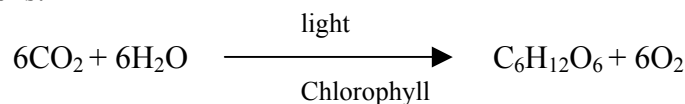
All fruit and vegetables are the end product of photosynthesis by the action of light on chlorophyll where inorganic carbon dioxide and water is transformed into organic compounds, specifically carbohydrates. The scale of photolysis and carbon dioxide fixation is phenomenal, it has been estimated that the photosynthetic process consumes 200 billion tons of carbon dioxide every year which is fortunate as it supports all life on planet earth.

This process of carbon fixation is a cycle and known as the Calvin cycle, the raw materials being sunshine, carbon dioxide water and of course essential minerals from the earth.

Figure 1 The Calvin Cycle



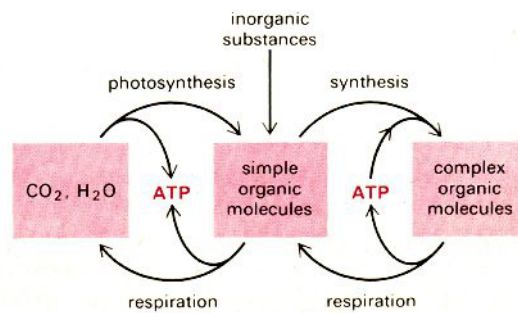
Overall the net results is:



## Fruit and Vegetable Metabolism

Photosynthetic production is just one of the steps in producing biomass, in fact during the day carbohydrate and oxygen is produced but during the night respiration occurs in order to build up the complex organic molecules required. The creative process of metabolism are usually referred to as anabolism and the destructive processes as catabolism, the null zone is known as the compensation point.

**Figure 2 Relationship between Anabolism and Catabolism**



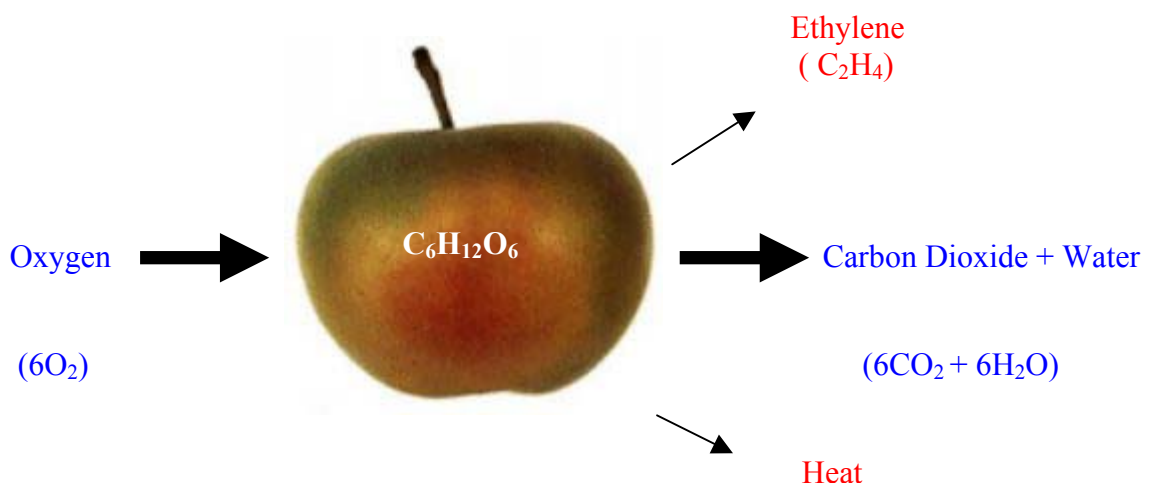
## Post Harvest

After harvest virtually no anabolic activity can occur as the fruit or vegetable has been detached from its food supply and chlorophyll remaining is insignificant. Therefore the only activity is catabolic which will eventually result in ripening, senescence and death of the organism but usually leaving the seeds for the next generation.

## Catabolic Activity

As described fruit and vegetables are alive; their cells are catabolising, consuming oxygen and carbohydrate and producing water vapour and carbon dioxide.

**Figure 3 The Storage Environment**



It can be seen the main products of catabolic metabolism are heat, water and carbon dioxide. Also note small amounts of ethylene gas are produced which is a plant hormone and can affect ripening and hence overall rates of metabolism.

## Factors Affecting Storage Life of Fruit and Vegetables

The storage life of fruit and vegetables is highly dependent on a number of factors listed below. This relates to their physiology, metabolic rate and is influenced by initial quality, temperature, relative humidity and gas concentration.

Success in extending the storage life depends on an understanding in the factors which directly effect this life. They are as follows:

**Pre-Harvest Conditions**      Top quality damage-free produce  
Minerals

**Temperature**                      Pre-cooling  
Correct storage temperature

**Controlled Atmosphere**      Physiology  
Relative humidity  
Oxygen  
Carbon dioxide  
Ethylene

Dealing with these factors in turn:

### Pre-Harvest Conditions

Top quality damage-free produce is essential for the successful storage life of fruit and vegetables. Growing conditions are important as shown in figure 2. Mineral inorganic components play an essential role in providing cell wall material, calcium being one of the most important.

### Minerals

It is fairly routine to treat apple orchards with calcium to prevent bitter pit in apples. However, this does appear on its own to have any affect on firmness. It has been shown that the addition of copper to the calcium treatment makes a significant difference to firmness and therefore keeping quality.

	Control	CA(OH) <sub>2</sub> 3g/litre	CU(OH) <sub>2</sub> + CU(OH) <sub>2</sub> 3g/litre
Skin firmness (trial 1)	7.4	7.7	8.1
Flesh firmness (trial 1)	55.6	55.9	60.2
Skin firmness (trial 2)	4.9	5.0	5.4
Flesh firmness (trial 2)	49.1	48.8	54.6

In this particular trial russetting of the fruit was a considerable problem, even though a significant enhancement of firmness was achieved in practice. In this instance the treatment would not be commercially viable.

Overall, the effect of calcium on plant senescence and fruit ripening is one of delaying and depressing the rate of senescence. The role of calcium within the metabolic process has been related by researchers to Calmodulin, a calcium binding protein has been shown to mediate the biochemical action of calcium in plants. The calcium calmodulin complex appears to activate several plant enzymes, thereby mediating the calcium signal and regulating cellular metabolism.

Calcium dips are often used commercially as a post harvest treatment but should be used with caution, treatment has been associated with induced skin injury. Fruit injury at the lenticells has been observed, including an observation of the tissue around the cell having a sunken appearance.

## **Temperature**

Temperature reduction to a point where neither chill damage nor freezing occurs is without doubt the most important factor associated with long term storage of fruit and vegetables. Each 10°C reduction in storage temperature results in a halving of the rate of metabolism. This can be compared with a reduction of metabolic rate of around 30% for produce stored in 2% oxygen from that which would exist at atmospheric concentrations of air.

## **Pre-Cooling**

As with all products shipped under temperature controlled conditions the importance of adequate pre-cooling must not be overlooked. It is easy to entirely negate the effects of CA by loading produce which is above the optimum carriage temperature.

## **Controlled Atmosphere**

Controlled atmosphere is extremely effective on apples and pears but less effective on other fruit where it generally gives a fifty- percent increase in shelf life. For CA to be effective, the fruit must have a strong climacteric or a high rate of metabolism.

Atmosphere control on a non-climacteric product such as a grape or cherry is not particularly effective. In common with other non-climacteric fruits, grapes do not ripen after harvest but are allowed to reach optimum maturity on the vine. After harvest the major obstacles to successful storage are water loss and the post harvest decay organism *Botrytis Cinerea*. Therefore high humidity and sulphur dioxide treatment are much more important than lowering the respiration rate. Similarly, weight loss and disease are more significant to cherry storage.

Controlled atmosphere storage of vegetables can be effective especially with quickly respiring shoots such as broccoli or asparagus, it being possible to reduce the rate of respiration by approximately 30%. However, this does not necessarily give a commensurate increase in storage life, as that would assume that respiration was the main factor in deterioration. In fact, respiration is only a minor factor, the major factor being decay pathogens whose action might be increased by the application of incorrect temperature.

The following table gives an indication of respiratory heat and whether the fruit is climacteric and therefore helps to indicate the likely effect of CA.

**Table 2**

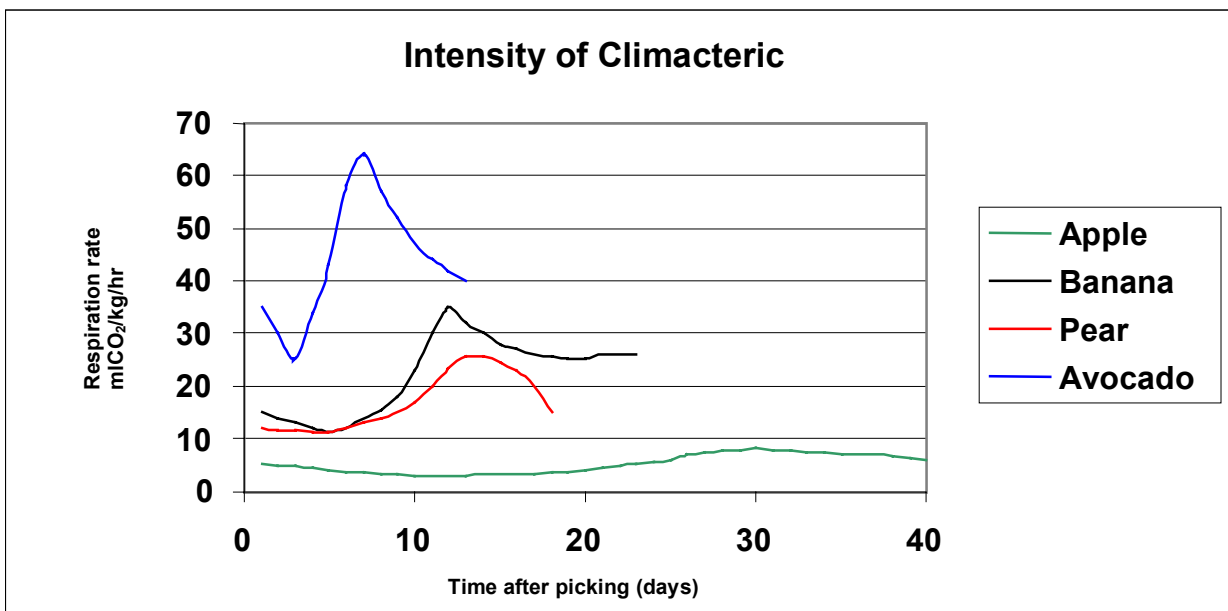
<b>Commodity</b>	<b>Respiratory Heat@ Normal Storage °C (Watts/tonne)</b>	<b>Climacteric</b>
Avocado	183-465	yes
Asparagus	81-237	no
Mango	133	yes
Banana	59-130	yes
Cauliflower	44	no
Apple	9-18	yes
Grape	3.9-6.8	no
Orange	18.9	no
Cherry	17-39	no

**Fruit Physiology**

To fully understand the benefits of controlled atmosphere we must look at the fruit physiology.

Fruit ripening is often accompanied by spectacular changes in respiratory rate, including a lowering of respiration in mature fruit followed by a large increase at ripening. After reaching the climacteric peak, respiration falls off as the fruit begins to enter senescent decline as shown below. The climacteric may be rapid as in avocados and bananas or at a slower rate as in pears and apples or non-existent as in citrus and grapes.

**Figure 4**



Most fruit will experience the climacteric whether or not they are picked. There is a blocking mechanism that tends to prevent ripening until detachment from the parent; this is especially strong in avocados which will not ripen until picked.

In many fruit, for example pears, the climacteric peak occurs at the time of optimal eating quality. In apples and bananas it just slightly precedes this optimal eating quality while in tomatoes it occurs well before the fruit is fully ripe.

## Relative Humidity

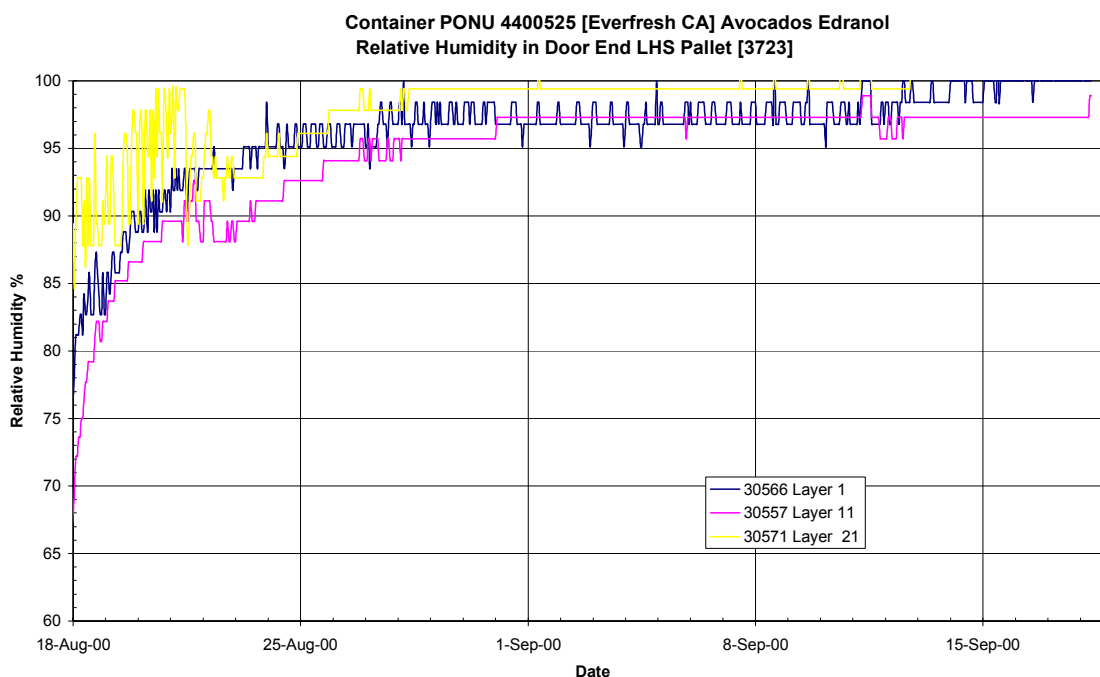
High relative humidity is essential for successful storage of products. Just a 2% loss of water is extremely detrimental to quality of the produce. The following shows the effect of differing storage humidities for kiwi fruit.

**Figure 5** Kiwi fruit after 12 weeks of storage at 0°C



Manufacturers have installed rh systems because of this requirement but operators have been reluctant to switch them on because of the risks associated with contaminated water. Results from recent trial carried out by CRT on a CA container with the rh control system switched off gave some surprising results.

**Figure 6**

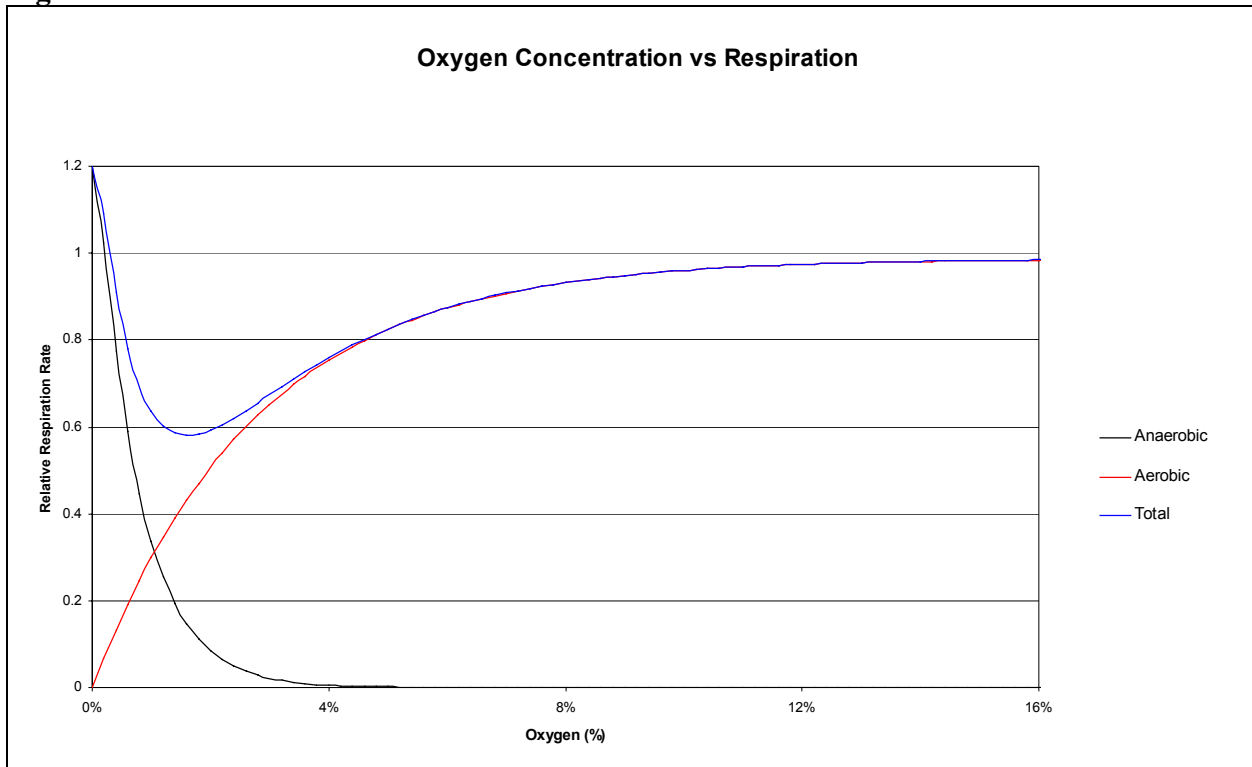


The graph shows that even though there is no water introduction or fresh air, the rh is high. This water must be the product of metabolism and transpiration.

## Oxygen Concentration

Reduction of oxygen concentration from 21% to 2% results in approximately a 30% reduction in metabolism. As the oxygen concentration decreases so does the metabolic activity as shown in the graph below for apples, measured in evolution of carbon dioxide.

Figure 7



Once the oxygen concentration gets lower than around 0.5%, the respiration rate actually increases, this is because anaerobic respiration has commenced which is less efficient and results in the formation of alcohol and acetaldehydes within the fruit. In extreme cases these can lead to tissue browning due to cell poisoning and even death of the of fruit or vegetable. Under anoxia, fermentive metabolism is induced and pyruvate is decarboxylated by pyruvate decarboxylase to produce acetaldehyde, which is then reduced to ethanol by alcohol dehydrogenase.

## Carbon Dioxide (CO<sub>2</sub>)

It is significant to storage requirements that the metabolic process evolves carbon dioxide and metabolic heat. Metabolic heat production is governed by product and temperature. The relationship between heat of metabolism and evolution of carbon dioxide is controlled by the relationship given below.

For every 2.96 Watts of metabolic heat 1g of CO<sub>2</sub> is generated. Accordingly, 44 grams of carbon dioxide occupies 22.4 litres of space at STP (standard temperature and pressure). Metabolic heat production can be taken from USDA or ASHRAE tables or the CRT Controlled Atmosphere Database.

Fruit can respond beneficially and all deleteriously to carbon dioxide in the storage atmosphere depending on the sensitivity of the tissue to the gas and its concentration, labour exposure, and temperature. Beneficial effects of carbon dioxide on fruit quality included decreased respiration



and ethylene production, delayed ripening, and the inhibition of decay and some physiological disorders. Deleterious effects included development of off flavours and carbon dioxide injury.

### Mechanisms of Carbon Dioxide Action

Respiration rates of fruit are usually reduced by elevated CO<sub>2</sub>, although stimulation of respiration can occur because of cell injury. CO<sub>2</sub> may affect enzyme activity by changing the rates of degradation and or synthesis, activation and/or inactivation, substrate and cofactor availability, or a combination of these processes. In addition CO<sub>2</sub> causes complex interactions in tissues, including changes in the kinetics of allosteric proteins.

Effects of CO<sub>2</sub> on enzyme activity are ascribed to the generation of carbonic acid and subsequent lowering of the intercellular pH. Theoretical considerations indicate this will occur at CO<sub>2</sub> concentrations above 5%.

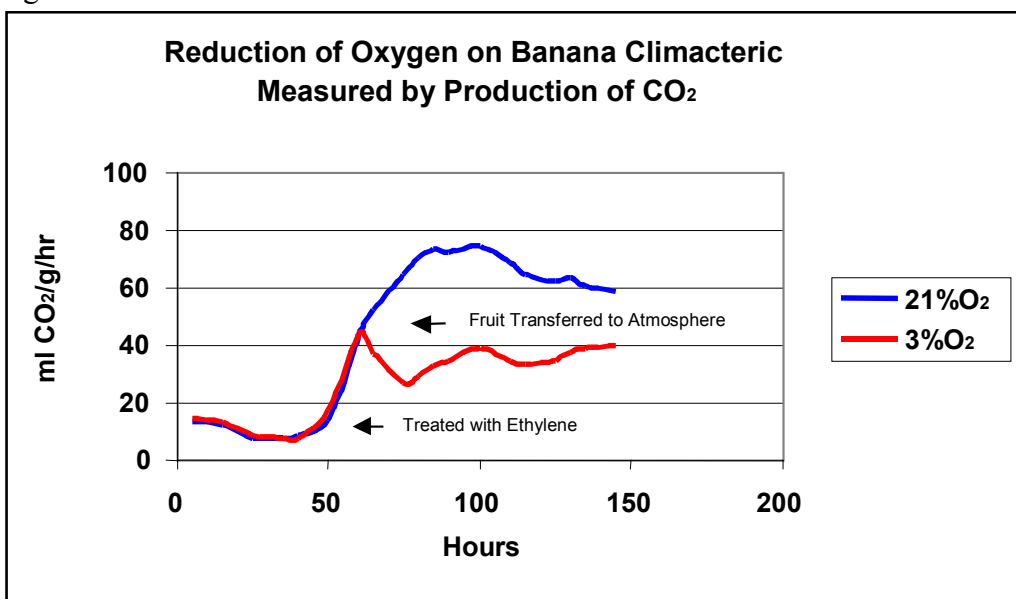
### Ethylene

Ethylene production is not linked directly to metabolic activity. This is because C<sub>2</sub>H<sub>4</sub> is a hormone and is used to trigger the ripening process or sometimes even as a response to injury. Volumes of ethylene, at least when compared to carbon dioxide production, are small. For example a high producer might generate in excess of 100 µlitres/h/kg of produce while a low producer might produce 0.1 µlitres/h/kg.

Carbon dioxide can be an inducer to or suppresser of ethylene production, depending on the commodity, the cultivar and its physiological age. CO<sub>2</sub> regulates ethylene biosynthesis in part by counteracting ethylene action. This is mainly by regulation of the enzymes 1-amino-cyclopropene-1-carboxylic acid (ACC) synthase, and also by ACC oxydase activity differentially regulated, being stimulated at low levels and inhibited at high levels. The final step in the biosynthesis of ethylene involves the catalytic conversion of (ACC) to ethylene by the ACC oxidase.

The effect of ethylene as a powerful ripening hormone and the effectiveness of CA in reducing respiration after initiation of the climacteric is shown below.

Figure 8



## Storage Life after Controlled Atmosphere Storage

It has often been suggested that once fruit is removed from a CA environment the metabolism speeds up because of the increase in oxygen. Therefore, the shelf life is shorter for a CA fruit than that of a younger piece of fruit of say for example the same equivalent firmness.

The following data from McDonald *et al*, demonstrates that effect for kiwi fruit stored in CA and then air.

Firmness of kiwifruit after initial cool-storage in air or in 5% CO<sub>2</sub> plus 2% O<sub>2</sub> at 0°C, followed by cool-storage in air at 0°C for 6 weeks and further 'shelf' storage in air at 20°C for 1 week.

**Table 2**

Year, and Storage Condition	after initial cool-storage in air or CA at 0°C	+	cool storage in air at 0°C for 6 weeks	+	'shelf' storage in air at 20°C for 1 week
Flesh firmness (kgf)					
1980 (1)					
Air	1.1		0.9		0.6
5%CO <sub>2</sub> +2%O <sub>2</sub>	4.7		2.2		0.9
1981 (2)					
Air	1.1		1.0		0.8
5%CO <sub>2</sub> +2%O <sub>2</sub>	2.5		1.8		1.0
1982(3)					
Air	1.5		1.2		1.1
5%CO <sub>2</sub> +O <sub>2</sub>	2.8		1.7		1.3
1.	Initial storage period = 20 weeks				
2.	Initial storage period = 12 weeks				
3.	Initial storage period = 8 weeks				

As can be seen in each of the years 1980 to 1983, the CA fruit was firmer than the air stored fruit after a period of "shelf storage".

## Flavour and Quality

With regard to flavour and quality, CA has been shown to usually maintain flavour though there can be both positive and negative effects.

In terms of flavour, good flavour is retained by slowing down the metabolism. Generally fruit and vegetables stored under controlled atmospheres have a longer storage life because the rate of the metabolic processes is slower. Particularly with climacteric fruit, CA slows ripening and deterioration so that when they have been stored for long periods they may well be less ripe than fruits stored in air. If the fruits stored in air are over-ripe or suffering physiological disorders associated with long storage, for example senescent breakdown, this would definitely affect the flavour negatively.

Negative effects of controlled atmosphere storage on fruit and vegetables metabolism can have other indirect affects on flavour, "off" flavours can develop if the oxygen is too low by means of anaerobic respiration. These chemicals, are usually alcohols and aldehydes would certainly affect the eating quality.

Storage can affect eating quality and there is evidence that fruit ripened after storage does not taste as good as fruit ripened directly after harvest. This is not always the case and depends on the length of storage and the type of produce. Generally flavour volatile compounds are less after storage than in freshly harvested fruit, but there is no evidence that controlled atmosphere storage affects flavour volatiles in any way that is different to air storage.

The acidity of the fruit and vegetables also has a significant effect on the flavour. Storage trials show widely different effects on acidity including levels of ascorbic acid (vitamin C). Generally the acid levels of fruit and vegetables should be greater after controlled atmosphere storage compared to air storage. This may be because carbon dioxide in the fruit is acid and the lower acid metabolism is associated with controlled atmosphere storage.

### **Safety of CA Products**

Other than the obvious of physically entering a CA space, there is little evidence of any dangers to health of CA products. The only conceivable risk is when modified atmosphere packaging is used where there could be a possibility of producing conditions favourable to botulism.

It has proven possible in the laboratory to develop an environment suitable for the Clostridium Botulinum organisms to be viable. For example if mushrooms were stored with too low a permeation rate of oxygen or at too high a temperature, all the oxygen could be consumed. In such a case the mushrooms would appear edible but could contain toxins injurious to health.

In practice this can be avoided by putting a few tiny holes in the packaging. This prevents the possibility of an ultra low oxygen environment developing but has minimal effect on storage life.

### **Conclusions and Summary.**

It has been shown that it is necessary gain a full knowledge of the product for which the storage life is to be extended. Maximum benefit can only be gained from controlled atmosphere storage if all of the criteria necessary for extended storage have been fulfilled. This encompasses growing conditions, minerals, water stress prior to harvest and post harvest satisfactory pre-cooling and storage temperature.

Controlled atmosphere systems have now reached a fairly advanced level of sophistication even compared to just two years ago at the last seminar. Nevertheless further advances can be made with regard to intelligent oxygen control measuring alcohol concentrations. There are possibilities for variable temperature regimes, gas concentrations and mineral additions to the humidity injection system or addition of say calcium by sublimation.

## **Glossary of Terms and Abbreviations**

### **ANAEROBIC RESPIRATION**

Respiration with insufficient oxygen present, normally producing alcohols and off flavours due to incomplete metabolic activity.

### **BITTER PIT**

Disorder associated with the pitting of the skin of apples, due to imbalance of minerals usually calcium in different parts of the fruit, as well as storage temperature, and levels of oxygen and carbon dioxide. The situation can be aggravated by cultural factors and climacteric conditions

### **C<sub>2</sub>H<sub>4</sub>**

Ethylene, a naturally occurring chemical which acts as a plant hormone to trigger physiological changes such as ripening, de-greening and leaf loss. Because it is a hormone its effect can be disproportionate to its concentration.

### **CA**

Controlled atmosphere usually implies the measurement and control of the normal atmospheric gases by some mechanically assisted system.

### **CHILLING INJURY**

Injury caused by low but non-freezing temperatures. Caused by damage to the metabolic pathways of the organism due to inability of enzymes to work below certain temperatures.

### **CLIMACTERIC**

A significant group of fruits show a variation from the normal respiratory pattern in that an increase in respiration coincides with ripening. This is known as a respiratory climacteric. Commercial maturity can be related to the rise in the respiration rate and/or ethylene production in climacteric fruits. As a general rule, for longest storage and optimum eating quality fruit should be picked just before the climacteric rise.

### **CO<sub>2</sub>**

Carbon dioxide, a gas which is normally present in the atmosphere at 0.03% and is a product of metabolism. It also is used to produce plant material by the process of photosynthesis.

### **CORE FLUSH/ BROWN CORE**

This disorder develops in several cultivars of apple during storage, as a brown or pink discoloration of the core. Usually caused by excessive levels of carbon dioxide.

### **DPA**

Diphenol aniline, a chemical used to treat apples to help prevent scald.

### **INTERNAL BREAKDOWN**

This disorder is characterised by an alcoholic odour and the taste of the fruit is tainted. Disorder is usually caused by failure to maintain recommended minimum oxygen levels or that the fruit has been stored too long at too higher a temperature.

### **MA**

Modified atmosphere usually implies the measurement and control of the normal atmospheric gases by some usually passive system such as special packaging.

**MAP**

Modified atmosphere packaging, as used above.

**METABOLISM**

Metabolism represents the entirety of the many chemical activities that occur within cells. The acquisition and storage of energy and the utilisation of this stored energy are two of the central processes in the control of the overall metabolism of plants.

**O<sub>2</sub>**

Oxygen, a gas which is normally present in the atmosphere at a concentration of 20.8%. It is required and consumed by metabolism and produces carbon dioxide and is a product of photosynthesis.

**RESPIRATION**

This is another word for metabolic activity and implies the consumption of gases by a biological process.

**rh**

Relative humidity, this is the percentage saturation of water vapour in air at any given temperature with 100% being fog. The ability of the air to hold water increases with increasing temperature and can be examined in detail by the use of a Psychrometric chart.

**RIBBON SCALD**

This disorder occurs on a few cultivars as a ribbon shaped brown band.

**SCALD**

Scald is a physiological disorder than can develop on apples and pears during storage and has been associated with ethylene levels in the store. Superficial scald in apples and pears is where the skin turns brown during storage.

**SKIN PITTING**

A light brown coloration showing through the skin of the fruit while it is still green

**VASCULAR STREAKING**

A disorder in cassava where the vascular bundles in the root turn a dark blue to black colour during storage. Disorder is associated with O<sub>2</sub> levels.

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