STORAGE LIFE ENHANCEMENT OF AVOCADO FRUITS

By

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ABSTRACT

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Avocado Fruit **(*Persea americana* Mill. Var. Hass)** is one of the most perishable commodities available in the market. It has a very high rate of postharvest respiration, limited shelf life and has special unique characteristics of ripening.

In the first part of this study, a silicon membrane system was used for the storage of 'Hass' avocado fruits. The silicone membrane system is an efficient method for attaining and maintaining modified atmosphere (MA) in experimental storage chambers.

The storage was performed in small sealed experimental chambers fitted with silicon membrane windows. The areas of the windows were calculated in order to achieve 3% oxygen assuming 30, 50, and 70% reduction of the respiration rate due to the effect of the modified atmosphere on the products' metabolic activity. Fruit stored at regular atmosphere (control) was kept under the same temperature (7°C) and relative humidity (90%) as those stored with silicon membrane system. The gas concentration in the chambers was analyzed using a gas chromatograph. The respiration rate was measured at storage (7°C) and ripening (15°C) temperatures. The effect of sulphur dioxide treatment on 'Hass' avocado fruit stored in the silicon membrane system was also evaluated.

Fruit quality before storage, after storage and after ripening was evaluated through physiological assessments. Fruit stored under the silicon membrane system remained in an excellent condition for 47 days. Following this period avocados ripened normally in a course of 4-10 days at 15°C and regular atmosphere. After ripening, the fruit did not show

any apparent physiological deterioration or damage, neither development of undesirable organoleptic changes. The chambers with the small membrane area reached stable gas concentration in 6 days, the chambers with the large membrane area never reached steady gas concentration to the desired levels; while the chambers with medium membrane area reached steady gas concentration in 15 days. Optimum results were obtained using small membrane area (28 cm^2 for a kilogram of stored avocado fruit) in the presence of sodium metabisulphate. This treatment has potential for commercial use after pilot scale studies.

In the second part of this study, observations were made on the effects of several plant regulators, 2,4-dichlorophenoxyacetic acid, gibberellic acid, and 6-benzylamino purine on the respiration pattern, ethylene production, and the number of days to ripen the avocado fruits. These substances were vacuum infiltrated to insure good penetration and distribution. The results indicated that these hormones inhibited the rate of degreening and softening. The metabolic activity of the treated fruit, as judged with the respiration rate, was diminished by using 6-benzylamino purine, it was more effective than the other hormones in reducing respiration rate throughout the ripening period. Stimulation of ethylene synthesis by hormone application was observed; however, enhanced ethylene evolution could not overcome the inhibition of degreening, softening, and the respiratory activity by the phytohormones. The results reinforce several previous observations with other fruits that auxins, gibberellins, and cytokinins may largely constitute 'resistance to ripening' and may be responsible for the lack of ripening shown by unpicked fruits.

RÉSUMÉ

L'Avocat est un des fruits les plus périssables disponible sur le marché. Il a un taux de respiration post-récolte très élevé, une durée de vie limitée sur les étagères et possède des caractéristiques spéciales et uniques de véraison.

Dans la première partie de cette étude, une membrane de silicone a été utilisée pour conserver les fruits d'avocat de la variété Hass.

La conservation a été effectuée dans petites chambres expérimentales scellés avec des ouvertures cuovertes par membranes de silicone. La surface des fenêtres ont étés dimensionnées afin d'atteindre 3% d'oxygène assumant 30, 50 et 70% de réduction du taux de respiration dû à l'effet de l'atmosphère modifiée sur le métabolisme du produit. Les fruits conservés dans une atmosphère non modifiée, le groupe de contrôle, étaient conservés sous la même température, soit 7°C, et une humidité relative de 90% comme ceux conservés avec le système à membrane de silicone. La concentration interne des gaz de la chambre a été analysée par chromatographie en phase gazeuse. Le taux de respiration a été mesuré à la température de conservation (7°C) et de véraison (15°C). L'effet d'un traitement au dioxyde de soufre sur les fruits d'avocat de la variété Hass conservés à l'aide du système a membrane de silicone a aussi été évalué.

La qualité des fruits avant entreposage, après entreposage et après véraison a été évaluée par échantillonnage. Les fruits entreposés avec le système à membrane de silicone ont conservés une excellente condition pour 47 jours. Après cette période les fruits d'avocats maturent normalement dans une période de 4 à 10 jours à une température de 15°C dans une atmosphère normale. Après véraison les fruits n'ont aucun signe de détérioration physiologique ou dommage et n'ont plus de développement organoleptique indésirable. La chambre avec les membranes de petite surface a atteint la concentration désirée de gaz en 6 jours, la chambre avec les membranes de grande surface n'a pas atteint la concentration désirée de gaz et la chambre avec les membranes de moyenne surface ont atteint la

concentration désirée de gaz en 15 jours. Le résultat optimal a été obtenu en utilisant les membranes de petite surface, soit 28 cm^2 par kilogramme d'avocats conservés, en présence metabisulfate de sodium.

Dans la deuxième partie de l'étude, des observations des effets de plusieurs régulateurs de plantes, soit l'acide 2,4-dichlorophenoxyacetique, l'acide gibberellique et 6-benzylamine purine sur le cycle de respiration, la production d'éthylène et le nombre de jours pour rendre le fruit d'avocat mure. Ces substances ont été infiltrées par vacuum pour garantir une bonne pénétration et distribution de ces substances. Les résultats indiquent que ces hormones ont inhibé le taux de déversdissage et de ramollissement. L'activité métabolique des fruits traités, à n'en jugé par le taux de respiration, était diminuée en utilisant le purine 6-benzylamino qui était plus efficace que les autres hormones à réduire le taux de respiration (troughout) la période de véraison. La stimulation de la synthèse d'éthylène par une application d'hormone a été observée, cependant l'évolution de l'éthylène activé n'a pas pu surpasser l'inhibition du déversdissage., du ramollissement et de l'activité respiratoire par les phytohomones. Les résultats renforcent plusieurs observations faites sur d'autres fruits indiquant que l'auxins, le gibberellins et les cytokinins pourraient constituer une résistance à la véraison et pourraient être responsables du manque de véraison observé sur les fruits non- récoltent.

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I cannot end without thanking my family, on whose constant encouragement and love I have relied throughout my life. I am especially thankful to my father, Luis Guillermo Forero. His unflinching courage and conviction will always inspire me. It is to them that I dedicate this work.

THESIS FORMAT

This thesis is submitted in the form of original papers suitable for journal publication. This thesis is prepared in manuscript format in accordance with the Part C of the "Guidelines for Thesis Preparation".

The thesis starts with a general introduction in chapter I to state the background of this project. In Chapter II, general objectives are provided. In Chapter III a literature review is provided. Chapters IV and V are manuscripts. The manuscripts are linked via connecting statements. A general conclusion and recommendation for future work is presented in Chapter VI. All the references cited are listed in the References Section of the thesis.

The work reported here was performed by the candidate and supervised entirely by Dr. G.S.V. Raghavan of the Department of Bioresource Engineering, Macdonald Campus of McGill University, Ste-Anne de Bellevue. The authorships for the papers in Chapters IV and V respectively are 1).M.P. Forero and G.S.V. Raghavan; and 2).M.P. Forero, G.S.V. Raghavan and D. Smith.

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NOMENCLATURE.

A	Membrane area.
a*	Chromacity coordinate (redness or greenness).
b*	Chromacity coordinate (yellowness or blueness).
CA	Controlled atmosphere.
CO_2:C_2H_4	Carbon dioxide to ethylene selectivity ratios.
CO_2	Carbon dioxide.
CO_2:O_2	Carbon dioxide to oxygen selectivity ratios.
CRD	Completely randomized design.
GA_3	Gibberellic acid.
GC	Gas chromatograph.
H_2SO_4	Sulphuric acid aerosol.
IAA	Indoleacetic acid.
IPA	Isopentenyl adenosine.
K_{CO2}	Permeability of the membrane for CO_2.
K_{O2}	Permeability of the membrane for O_2.
L*	Chromacity coefficient (lightness).
LSD	Least square difference.
m	Mass of product to be stored.
MA	Modified atmosphere.
$Na_2S_2O_5$	Sodium metabisulphate.
O_2	Oxygen.
PP_{CO2}	Partial pressure difference of CO_2 across the membrane.
PP_{O2}	Partial pressure difference of O_2 across the membrane.
RA	Regular atmosphere.

RR_{CO2}	Respiration rate for CO_2 produced.
RR_{O2}	Respiration rate for O_2 consumed.
SO_2	Sulphur dioxide.
T1	Treatment 1: (2,4-dichlorophenoxy-acetic acid).
T2	Treatment 2: (gibberellic acid).
T3	Treatment 3: (6-benzylamino purine).
T4	Treatment 4: (D-mannitol).
T5	Treatment 5: (water).
T6	Treatment 6 (evacuated non infiltrated fruit).
T7	Treatment 7 (Control fruit).
2,4-D	2,4-dichlorophenoxy-acetic acid.
6-BA	6-benzylamino purine.

CHAPTER I

GENERAL INTRODUCTION.

Despite the remarkable progress made in increasing food production at the global level, approximately half of the population in the Third World does not have access to adequate food supplies. There are many reasons for this, one of which is food losses occurring in the postharvest and marketing system. Evidence suggests that these losses tend to be highest in those countries where the need for food is greatest, as their economy is poor.

In general fruits and vegetables are quickly perishable, and if care is not taken in their harvesting, handling, and transport, they will soon decay and become unfit for human consumption. Estimates of production losses in developing countries are hard to judge, but some authorities put losses of sweet potatoes, plantain, tomatoes, bananas and citrus fruit sometimes as high as fifty percent of what is grown. Reduction in this wastage, particularly if it can economically be avoided, would be of great significance to growers and consumers alike.

There has been significant growth in the international trade in tropical and subtropical fruits, which satisfy a demand for variety of luxury in regions where they cannot be grown. The international trade in fruits such as banana and citrus fruits has been well established for several decades and these fruits are imported/exported in large volumes in contrast to lesser-known tropical fruits. Lack of acreage expansion and accessibility to foreign markets for many of these lesser-known tropical and subtropical fruits has been caused by restrictive climate and growing conditions; ineffective or poor agricultural techniques; limited postharvest knowledge for harvesting, handling, and transporting; lack of agribusiness initiative to stimulate production, marketing, and research; and lack of knowledge of food value and functionality of processed products. For fruit with a well established market, a considerable body of research data and of practical commercial

experience enables these commodities to be handled, packaged, transported and distributed to ensure that good quality produce is delivered to the consumer at a lesser cost.

Advanced cultural and production practices coupled with improved fruit cultivars have contributed markedly to superior fruit quality at harvest as well as increased yields. Unfortunately, the impact of these improvements has not been fully realized for many fruits and vegetables at the wholesale, retail, and consumer levels because of spoilage. This situation is further aggravated by the fact that although these produce may be of marketable quality, they are often plagued with unfavourable marketable characteristics by the time they reach the consumer.

Storage of fruits and vegetables is practised for various reasons. It is part of orderly marketing, where the storage period is usually short, to allow for the accumulation of sufficient produce by a grower or group of growers to send it to the market. It may be stored in wholesale markets during the period it is being sold. Also it may be stored when the price at a particular time is low, to await an increase in price. Certain crops are stored for long periods of time to extend the duration of their availability. In this latter case the crop is grown purposely for long term storage, and even specific cultivars are grown. However, long term storage can be expensive and require a high level of technical knowledge of the cultivar or variety of the crop and the appropriate storage conditions.

The significance of ethylene in ripening was established early in the twentieth century and is the centre of postharvest and physiological research. Of far greater significance to the consumer are the other changes that occur during ripening, including softening, colour changes, sugar content, flavour, and aroma. As the tissues of stored fruits and vegetables are still alive, they require environmental conditions which will lead to retarded maturation, so that the appearance, colour, flavour, texture, aroma and other qualities for which they are prized, will be preserved. It is not possible to improve the quality of a produce after harvest, but it is possible to reduce the rate of quality loss. This is the key concept in horticultural produce storage. Maintenance of quality is highly dependent on the development of

adequate postharvest technology for serving these ends. Although many techniques have been developed, a great portion of the research is still to be carried out in fruit physiology. Research in fruit physiology and postharvest technology are dependent on each other for storage attempts to be successful.

Studies on the physiology of fruit preservation must be carried out on an experimental basis because there are no guidelines that can precisely identify optimum storage conditions for a single product. This is because the relative rates of the biochemical and physiological changes occurring are subject to several factors. The importance of each change and the rate at which it occurs may differ between cultivars, depend on maturity and temperature or vary according to climate and soil conditions in which fruits are grown.

CHAPTER II
GENERAL OBJECTIVES.

This study has the general objective of evaluating different treatments on 'Hass' avocado fruits to enhance their postharvest life.

This overall objective can be represented by four main objectives, which consist of:

1. Enhancing the storage life of 'Hass' avocado fruits through modified atmosphere (MA) storage.
2. Determining the effects of sulphur dioxide as a chemical treatment on stored 'Hass' avocado fruit.
3. Testing and comparing the effects of three growth regulators on ripening 'Hass' avocado fruits that were previously infiltrated through vacuum.

CHAPTER III
LITERATURE REVIEW.

3.1. General Introduction on Avocado.

Native to the Western Hemisphere from Mexico south to the Andean regions. The avocado tree (*Persea americana* Mill.) belongs to the family of the Lauraceae. Other members of the genus are known but they do not appear to be commercially important. Avocados were widely cultivated in tropical America as individual seedling trees before the Spanish conquest but did not receive serious horticultural attention until about 1900, when horticulturists found that production of grafted trees was simple and allowed perpetuation of superior seedlings and the establishment of orchards. There are three general cultivars namely the Mexican, Guatemalan and West Indian, each of which comprises a group of varieties. Apart from these, there are many important hybrids. The well known variety 'Fuerte' is a Guatemalan-Mexican hybrid, while 'Hass' is of Guatemalan origin. There is a great variability in fruit traits not only between races but between cultivars within a race. One of the most distinct differences between cultivars is the peel colour when ripened. The peel of some cultivars changes from green to black or purple with increasing maturity or ripening. The names of the cultivars reflect the area of origin of the avocado, and fruit properties such as ripening, quality and abscission are related to the climates of the respective areas.

3.1.1. World market and fruit characteristics.

In the developed countries, avocados tend to be a luxury item and the relatively sophisticated growers, consumers, marketing and government agencies have been the driving forces behind maturity investigations. In the developing countries of Latin America, however, avocados are a staple food and the relative unsophistication of the consumers and

the other groups, or perhaps the greater consumer awareness of avocados, has reduced the pressure for maturity standards. This situation may be changing as urban population increase in developing countries, and exports become important.

The avocado has a long history as both a subsistence and marketable fruit in the areas of origin in Central and South America. Recently, however, a considerable trade has developed, both locally and internationally, with the fruit becoming well known in industrialized areas of North America and Europe. Flourishing industries developed in Florida and California, in South Africa, and on a somewhat smaller scale in Chile, Brazil, Hawaii, Australia, and some islands of the Pacific. Mexico, where avocados are extremely popular, produces large quantities annually; commercial plantings have been made in Israel, and there are numerous trees in other countries around the Mediterranean.

Mexico is the world's leading producer of Hass avocados. In fact, if you combine the production of the next four countries it would still not even come close the annual production of Mexico. World production of avocados was 2.4 million tons in calendar year 2001, an increase of less than one percent from the previous year. Mexico led the world by producing 940,000 tons, 40 percent of the world total. The United States was the second largest producer with 164,500 tons. Other countries in the top five included Indonesia (130,000 tons), Chile (120,000 tons) and Peru (89,800 tons).

Total export of avocados in 2000 reached 310,671 tons, up 13 percent from 1999 and 15 percent from 1998. The top exporters in 2000 were Mexico (29 percent), Chile (18 percent), South Africa (16 percent), Israel (14 percent) and Spain (14 percent). Other key exporters included France and the Netherlands.

In 2000, France led the world by importing 105,249 tons of avocados, 31.2 percent of total world imports of avocados that year. Key exporters to the French market include Israel, Spain and South Africa, comprising 35, 24 and 15 percent of the market, respectively. The United States imported 78,533 tons, the second largest amount in 2000. Other key importers included the Netherlands, United Kingdom, Japan and Canada. Table

3.1. shows avocado production, supply, and distribution in some countries for the years 1997/98 – 2001/02.

One probable reason for the increase in avocado popularity is the high nutritional value of the fruit. Table 3.2. shows some characteristics of avocado in terms of minerals and vitamin contents in the pulp. The avocado fruit has a high protein, fibre, and vitamin (A, C, and E) content; the sugar level is relatively low when compared to other fresh fruits. It is an excellent source of potassium and phosphorus, and contains mono-unsaturated fatty acids which effectively reduces the levels of low density lipoproteins in the blood (cholesterol), helping in the prevention of coronary diseases. The avocado oil expressed from the flesh is rich in vitamins A, B, C, and E. It has a digestibility coefficient of 93.8% but has remained too costly to be utilized extensively as salad oil. The amino acid content has been reported as: palmitic (7%), stearic (1%), oleic (79%), and linoleic (13%) (Morton 1987).

Table 3.1: Avocados: Production, supply and distribution in selected countries. Marketing Years 1997/98 – 2001/02 (Metric Tons).

Country/ Marketing Year 1/	Production	Imports	Total supply	Exports	Fresh domestic consumption	Total distribution
Chile						
1997/98	86,500	0	86,500	44,514	41,986	86,500
1998/99	80,550	0	80,550	34,787	45,763	80,550
1999/00	95,000	0	95,000	52,049	42,951	95,000
2000/01	98,000	0	98,000	52,500	45,500	98,000
2001/02 F	110,000	0	110,000	60,000	50,000	110,000
Israel						
1997/98	64,000	0	64,000	35,000	26,000	64,000
1998/99	46,000	0	46,000	26,000	18,000	46,000
1999/00	77,000	0	77,000	45,900	29,100	77,000
2000/01	63,000	0	63,000	38,000	23,000	63,000
2001/02 F	70,000	0	70,000	44,000	24,000	70,000
Mexico						
1997/98	762,336	0	762,336	34,117	714,486	762,336
1998/99	550,000	0	550,000	38,571	493,429	550,000
1999/00	876,623	0	876,623	22,415	809,208	876,623
2000/01	898,168	0	898,168	52,475	800,693	898,168
2001/02 F	970,000	0	970,000	70,000	855,000	970,000
South Africa						
1997/98	100,000	0	100,000	52,000	38,000	100,000
1998/99	65,000	0	65,000	33,000	24,000	65,000
1999/00	104,000	0	104,000	54,000	38,000	104,000
2000/01	80,000	0	80,000	36,000	35,200	80,000
2001/02 F	100,000	0	100,000	45,000	46,000	100,000
Spain						
1997/98	60,000	3,560	63,560	54,878	8,682	63,560
1998/99	73,000	2,650	75,650	44.900	30,750	75,650
1999/00	58,000	3,600	61,600	46,300	15,300	61,600
2000/01	47,000	4,500	51,500	39,400	12,100	51,500
2001/02 F	60,000	5,000	65,000	45,000	20,000	65,000
United States 2/						
1997/98	161,706	47,775	209,481	4,230	205,251	209,481
1998/99	144,469	55,539	200,008	6,060	193,948	200,008
1999/00	166,287	66,214	232,501	3,454	229,047	232,501
2000/01	212,572	76,650	289,222	1,712	287,510	289,222
2001/02 F	210,000	75,000	285,000	3,500	281,500	285,000

Import and export data from Census Bureau, Department of Commerce. F = Forecast. Sources: Reports from USDA/Foreign Agricultural Service (FAS) attached reports, USDA/NASS estimates, and U.S. Department of Commerce (World Horticultural Trade & Export Opportunities Feb. 2002).

Table 3.2: Composition and nutritional value of avocado per 100g of edible portion.

I. Ingredient	Content
Moisture	65.7-87.7g
Ether extract	5.13-19.80g
Fiber	1-2.1g
Nitrogen	0.13-0.38g
Ash	0.46-1.68g
Potassium	400mg
Phosphorus	25mg
Magnesium	18mg
Calcium	7mg
Sodium	6mg
Copper	0.16mg
Iron	0.5mg
Manganese	0.08mg
Ascorbic acid	13mg
E	3mg
Niacin	1.45mg
Pyridoxine	0.45mg
Riboflavin	0.21mg
Carotene	0.13mg
Folic acid	0.18mg
Thiamine	0.08mg

Source: Morton (1987)

3.1.2. Physiology.

Avocado is prized for its rich nutty flavour and for its high oil and protein content. Though fruit intended for storage (20 to 25 days, e.g., 14 to 18 days in cold storage during overseas transport, and seven to ten days for ripening and distribution) in cold is picked in a firm and inedible condition, nevertheless it must have reached a stage of maturity which will permit a normal ripening. Fruits picked before this stage of maturity has been reached behave abnormally. Fruit which has become almost ripe on the tree, on the other hand, will only remain in good condition during a relatively brief storage period (Wardlaw, C.W. 1937).

The avocado differs from most other fruits in that ripening does not normally take place on the tree, but only after picking. Normal avocado softening with acceptable taste occurs only when a certain level of maturity, has been reached. Before this state of maturity is reached, only slight softening may occur due predominantly to shrivelling as a result of water loss, and flavour is poor. Once horticultural maturity has been reached, the rate of postharvest softening becomes progressively shorter with increasing maturity.

The avocado has a high rate of postharvest respiration and limited shelf life. The avocado fruit is climacteric, which implies a marked rise in respiration rate on the onset of ripening, followed by a decline.

The elevated production of ethylene, by even several ripe fruits in a shipment, may trigger a chain reaction, causing premature ripening of the whole load (Peleg et al., 1990). Although the fruit is usually considered as an organ which ripens quite uniformly, Tingwa and Young (1975) in their studies with the 'Hass' variety reported that ethylene production and softening of the fruit were always detected earlier in the stem end than in other parts of the fruit. Further experiments carried out by Adato and Gazit (1977) also show a distinct difference in ripening rate between different parts of the avocado fruit, their results demonstrate the difference in the course of softening between the fruit parts. Softening started at the distal (the part below the seed) part of the fruit and was consistently delayed at the middle part and specially at the proximal (the part between the seed and the pedicel) part of the fruit.

The most obvious feature of avocado fruit ripening is softening; firmness differences in avocado fruits are good predictors of the difference in their ripening stages, since the softer fruit will fully ripen, sooner than the harder fruit. The most common physiological parameters for determining avocado ripening are ethylene evolution and respiration rate (Pesis et al., 1994).

A number of methods or combinations thereof are available for decreasing the rate of ripening, and they are used for avocados; however, the combination of high metabolic

activity and their susceptibility to chilling injury means that care is necessary in the choice of storage conditions. The ongoing research evident in the literature is an indication that problems have not been solved satisfactorily yet.

3.2. Storage Systems for Avocado Fruit.

Storage systems can be classified as natural and artificial. The natural storage system consists of leaving the product in the field without any additional care. The artificial storage systems give the product ideal conditions to keep it in the most desirable quality form for the longest possible time. The shelf life can be prolonged throughout treatments such as postharvest disease control, atmosphere regulation, chemical treatments to delay or prevent sprouting or affect the crop's metabolism, application of waxes and other coating materials, irradiation, and refrigeration. Refrigeration is the one in which better results have been shown in the storage of fruits and vegetables, the other methods are efficient only when they are complemented with low temperatures.

Low temperature storage is the most commonly used method of extending storage life in avocado. The extent to which avocado can be chilled depends on the cultivar, temperature of storage, and period of storage.

The use of storage conditions containing high concentrations of carbon dioxide and low concentrations of oxygen coupled with low temperatures has proven successful in delaying ripening and senescence in many fruits. Low levels of oxygen decrease overall respiration rate, and also appear to block the ethylene-forming system. High carbon dioxide concentrations also decrease ripening, possibly by acting as a competitive inhibitor of ethylene.

Hypobaric storage has received attention. Ahmed and Barmore (1980) quote work by a number of authors, showing that avocados can be stored successfully for long periods under low pressure. The success of hypobaric storage appears to be in the increased diffusion of ethylene from the fruit, thus lowering internal concentration. The lower partial

pressure of oxygen may also play a role. The use of hypobaric storage in practice is limited because of practical problems similar to those with controlled atmosphere storage.

Various other treatments can be applied to avocado fruit to enhance storage life. The most commonly used postharvest treatment is that of waxing the fruit. In addition to conserving moisture, waxing is also believed to modify the internal atmosphere of the fruit tissue, decreasing the internal oxygen and increasing carbon dioxide concentrations (Durant et al., 1984), which will retard ripening (Rhodes 1981).

While an alteration in the normal atmosphere surrounding the fruit may have positive effects during storage, incorrect ratios of oxygen and carbon dioxide may result in physiological disorders. While many aspects of the initiation of avocado physiological disorders are still unknown, and of the known factors, the relative influence of each is unclear. Membrane structure, function, and stability under stress together with the postharvest conditions of temperature, humidity, oxygen, carbon dioxide, and ethylene, to which the fruit is exposed, are all important.

3.2.1. Refrigeration.

When intended for export, Avocado fruit may be harvested relatively immature, transported at a suitably low temperature (which retards but does not entirely arrest maturation processes) and subsequently ripened at a higher temperature. During cold storage of Avocado fruits even at relatively low temperatures, maturation processes slowly continue so that fruits ultimately become ripe and finally over-ripe, Avocados may ripen fairly quickly even at a storage temperature of 10°C (Wardlaw 1937).

Temperature management is the most important tool for extending shelf life and quality of agricultural commodities (Olorunda 2000). For most avocado cultivars, there is a significant decrease in the self life with increased storage temperatures. Respiration is greatly suppressed in avocado stored at low temperatures. Table 3.3 shows the respiration

rate of avocado fruit and other climacteric and non-climacteric fruits at different storage temperatures.

The optimum temperature for storage of avocados has been shown to vary according to cultivar and the storage technique employed (Esguerra et al., 1992). Precise knowledge of the storage temperatures best suited to different varieties is essential to avoid chilling injury.

Chilling injury in avocados cause symptoms such as pitting, browning of pulp near to the seed or in the tissue midway between the seed and the skin, failure to soften when transferred to a higher temperature, off-flavour, vascular strands and development of brownish appearance. Table 3.4 shows the approximate storage temperatures at which chilling injury can occur.

Hawaiian avocados stored at 0 - 2°C resulted in chilling injury when stored for a long period of time but this injury did not appear in the first three to four weeks of storage (Higgins et al., 1911); Wardlaw (1937) stated that Hawaiian avocados could be stored green at 2.2°C for six to eight weeks with a two to five days of satisfactory ripening; while Wilcox (1914) reported that Hawaiian fruits could be held at 0°C for two months without injury. In 1930 (in California), it was a common practice to store avocado fruits in the range of 4.4 - 7.2°C (Wardlaw 1937); In the West Indies a set of experiments with a large number of local seedling varieties, it was found that only a few varieties could be held at a temperature of 7.2°C for 20 to 25 days without sustaining chilling injury. In a study carried by Zauberman *et al.,* (1977) with 'Hass', 'Fuerte' and 'Naval' fruits they reported that at 6°C and 8°C, the metabolic activity of the fruits was reduced and fruit ripening inhibited; the fruits did not soften until they were transferred to a higher temperature. Storage of the 3 cultivars at 6°C and 8°C did not cause any chilling injury for 6 weeks. The shelf life at 25°C after storage at these temperatures was shorter than for fruits with out previous cold storage exposure. 'Nabal' and 'Hass' were more resistant to chilling injury than 'Fuerte' fruits. This data refers to fruits that were placed in storage the day after picking, which

started the process of ripening and softening but responded differently (Kosiyachinda and Young, 1976).Chilling injury occurred in 'West Indian' seedling avocado fruits when they were stored at less than 13°C (Thompson *et al.,* 1971).

Chilling in avocados is a complicated phenomenon in which various factors are involved. Among others, the temperature and duration of storage are important; some varieties ripen normally after 20 days at 7.2°C on removal to a higher temperature, whereas some if held at 7.2°for additional 10 days chill effects develop (Wardlaw, 1937). The maturity of the fruit at the time of storage is also of critical importance. Fruits stored immature only show chill effects after long exposure to the low temperature; whereas fruits of the same variety stored when they are more mature may show chill effects in a relatively short time. In general, fruits are mostly subjected to chilling during the initiation of ripening.

Table 3.3: Effects of temperature on the respiration rates of selected fruits expressed in millilitres of carbon dioxide produced per kilogram per hour [mlCO_2/kgh].

II. Fruit	**0ºC**	**5ºC**	**7ºC**	**10ºC**	**13ºC**	**15ºC**	**18ºC**	**20ºC**
Avocado	-	10-25	-	25-80	-	62-157	-	40-150
Breadfruit	-	-	-	-	-	-	-	38-178
Banana	-	-	-	-	10-30	12-40	15-60	20-70
Cherimoya	-	-	-	25-100	-	45-150	-	75-250
Peach	2-3	6-9	-	8-12	-	32-34	-	32-55
Pineapple	-	2	-	4-7	-	10-16	-	28-43
Strawberry	6-10	-	-	25-50	-	-	-	50-100
Guava	-	-	-	4-30	-	-	-	10-70
Kiwi	1.5-2	5-7	-	9-12	-	3-4	-	15-20
Mango	-	-	-	12-16	15-22	19-28	-	35-80

Passionfruit	-	15-30	-	20-40	-	-	-	45-100
Papaya	-	-	3-5	4-6	7-9	10-12	-	15-35
Watermelon	-	-	2-4	3-5	5-8	8-10	-	-
Sapote	-	-	-	-	-	-	-	25-35

Source: California University, http://www.postharvest.ucdavis.edu/produce/producefacts/

Table 3.4: Effects of storage temperature on time from harvest to softening and chilling injury symptoms of seedling avocados.

Storage temperature (ºC)	Mean days to softening	Chilling injury % affected fruit
7	16	23
10	13	16
13	16	27
18	9	0
27	8	0

Source: Thompson et al., 1971.

3.2.2. Controlled (CA) and modified atmosphere (MA).

Modified atmosphere refers to holding produce under conditions of atmosphere modified by package, over wrap, box liner or pallet. Oxygen is reduced through respiration by the produce, and the carbon dioxide level is determined by the permeability of the film, respiration, temperature, tightness of the transport vehicle, and other factors (Raghavan et al., 2005).

The concept of modified atmosphere was developed almost simultaneously with that of controlled atmosphere storage, it is a physical technique that does not leave chemical residues on the food and is referred to any atmosphere in which gas content is different from regular atmosphere. Here carbon dioxide and oxygen levels are not controlled to

specific concentrations. A controlled atmosphere refers to an atmosphere with a strict control of the gas concentrations of oxygen, carbon dioxide, and nitrogen; generally, the concentration of oxygen gas is lower than that of carbon dioxide and nitrogen concentrations are higher than that of regular atmosphere.

The first storage of avocados under conditions of low oxygen was carried out in Los Angeles in the University of California, 1940. Commercially, the first storage of avocadoes under controlled atmosphere was done in Florida (Spalding and Reeder 1974a). Storage under controlled atmosphere doubles the shelf life of the avocados when compared to storage under regular atmospheres; this allows commercializing the commodity throughout the year (Raghavan et al., 2005).

By using subnormal concentrations of oxygen alone, or in conjunction with high concentrations of carbon dioxide, a definite deceleration can be induced in the maturation of avocados. The range of tolerance to such treatments, however, varies from variety to variety, being comparable though not necessarily parallel to the response of different varieties to low temperatures. In varieties unsuited to gas storage, external and internal damage may be sustained without the intervention of microorganisms. In other varieties, although no direct physiological injury may be apparent, the fruits subsequently prove much more susceptible to the inroads of storage pathogens. Others, again, show surprising tolerance of gas storage conditions and would undoubtedly lend themselves to commercial preservation by this method. It is interesting to note that in this category there are varieties subject to chilling and consequently unsuitable for transport at low temperatures (Wardlaw, 1934).

Overholser (1928) reported good quality of ‘Fuerte’ avocado fruit stored for two months at 7.2°C under a 4-5% oxygen and 4-5% carbon dioxide atmosphere, whereas the same variety stored at regular atmosphere and 21°C can only be stored for 10 days, and for one month at 7.2°C.

3.2.2.1. Membrane system.

Membrane system is a type of modified storage which has been designated as self-controlled atmosphere system. Sealed polyethylene sacks of a defined thickness with fixed ratios of weight of fruit to area of sack surface are used. The theory is that when the right film and right temperature are used, the sack will maintain a theoretically correct and beneficial mixture of carbon dioxide and oxygen. The method was invented by two French investigators (Marcellin and Leteinturier 1966, 1967); it involved the use of elastomers of silicone or membranes of dimethyl polysiloxanes (Smock, 1979). The membranes "breathe" oxygen and carbon dioxide, allowing for reduced oxygen and elevated levels of carbon dioxide. It is the selective permeability of the membrane to gases, combined with the respiratory activity of the product that permits the enrichment of carbon dioxide and the depletion of oxygen concentrations within the storage unit. In this type of storage unit, the product stored maintains its own atmosphere by the combined effects of the respiration process of the commodity, leakage of gas and diffusion rate through the membrane.

The basic concept was developed in France by Jacques Etienne Berard. His studies were conducted in 1819 and 1820 and published in 1821. Berard recognized that harvested fruits utilize oxygen and give off carbon dioxide. He observed that fruits placed in an atmosphere deprived of oxygen did not ripen as rapidly. It was a century later in the United Kingdom when scientific studies were initiated. The first experimental study to develop the technique into a technology was carried out in England in 1918 by Franklin Kidd and Cyril West (Bartram, 1996). These researchers found that by diminishing the concentration of oxygen or incrementing carbon dioxide concentration the metabolic activity of apples was reduced and the shelf life was extended.

The biggest commercial development occurred in the United States of America in the decade of 1950. Regardless of being a technique that can be used for many agricultural products, the first storage work was done with apples. Both producers and consumers keep

benefiting from this technology leading to superior quality of the fruit for a longer period of time. This is a situation where both groups benefit from a new agricultural technology.

When fruits are packed in polymeric films, the atmospheric composition within the package is altered by the respiration of the product (The atmosphere within a fruit is controlled by the respiration of the product, the production of ethylene, the natural permeability of the fruit to gases, and to the differential partial pressure in and out of the product) and the diffusion of the gasses trough the package. The composition, thickness, structure, superficial area of the package, as well as the temperature and the differences of partial pressure of the gases in and out, are the principal factors that contribute to the gas exchange trough the membrane.

The technique of using a package to generate modified atmosphere is widely used, individual packaging such as for citrus and guavas, boxes such as for plantain and banana, pallets for strawberries, or in containers for sea transportation.

Modified atmosphere package can be used in a passive way in which the modification of the atmosphere is done by the respiration of the packaged product. When the product characteristics and the permeability of the film are adequately combined, an appropriate atmosphere can be achieved in a passive way within a sealed package as a result of the consumption of oxygen and production of carbon dioxide through respiration (Zagory and Kader 1988).

In a study of West Indian avocado fruits storage (Thompson *et al.,* 1971) fruits stored in polyethylene bags at 13°C significantly delayed softening. Perforation of the polyethylene bags did not significantly increase storage life compared with that of unwrapped fruits. They concluded that wrapping West Indian avocado fruits in polyethylene bags and storing them at 13°C would generally prolong the storage life sufficiently to allow for transport by sea to northern industrial countries. Placing fruits individually in polyethylene bags reduces their water loss and the gaseous exchange with the outside atmosphere.

While an alteration in the normal atmosphere surrounding the fruit may have positive effects during storage, incorrect ratios of oxygen and carbon dioxide may result in physiological disorders. Spalding and Marousky (1981) found the critical oxygen level for damage to be approximately 1%. At concentrations lower than this anaerobic respiration occurs. Avocados can also be adversely affected by high carbon dioxide content, it is less critical, with concentrations of up to 25% provided oxygen is not limiting. Careful manipulation of the surrounding atmosphere can be beneficial in the prevention of physiological disorders.

Modified atmosphere storages may also reduce chilling injury, because low oxygen and high carbon dioxide partial pressures retards respiration and ethylene production (Spalding and Reeder 1975). Fruit stored at low temperatures had more chilling injury than fruit stored in air (Lee and Young 1984). Guatemalan and Mexican varieties held in 0.025mm gauge polyethylene bags contained 6.4% Carbon Dioxide and 7.8% Oxygen after 5 days in cold storage and 7.9% Carbon Dioxide and 5% Oxygen after 23 days (Aharoni *et al.,* 1973).

Packaging provides a barrier to gas exchange with the external atmosphere. The atmosphere provided by this barrier depends on the type of material, the velocity of the ventilating air around the product. A storage room or a storage place in a vehicle are also barriers. The atmosphere surrounding the product depends of how hermetic the room, package or vehicles are, and to the movement of air (ventilation) inside them. The effect of these barriers to the gas exchange is cumulative and must be considered when selecting an adequate handling condition for the product.

The utilization of modified atmospheres in transport vehicles is complicated because they are not hermetically sealed. Nevertheless, most of the train refrigerated units are relatively hermetic and allows the use of modified atmospheres. Modified atmospheres can also be used in containers for maritime transportation. In the past 20 years, in Mexico small quantities of avocado and mango have been transported under modified atmospheres

to Europe and Asia without major success, due to the inadequate and inefficient system followed and without an adequate control of the atmospheric composition. Other countries in Latin America, including Chile, have transported fruits under modified atmospheres following the same system used in Mexico.

The use of controlled atmosphere as storage system for perishable products usually requires high costs in both construction and maintenance of the equipment.

3.2.2.1.1. Silicon membrane system.

The silicon membrane system allows gases to diffuse at different rates according to their physical and chemical properties. With this system, the atmospheric composition is obtained through two simultaneous processes, membrane permeation and product respiration (Gariepy *et al.,* 1986).

Procedures to calculate the membrane area required are based on the level of carbon dioxide or oxygen desired in the storage chamber and can be calculated using Equations 3.1 and 3.2.

Equation 1. Area of silicon membrane for desired CO_2 concentration.

$$A = \frac{RR_{CO_2} \cdot m}{K_{CO_2} \cdot PP_{CO_2}} \qquad (3.1)$$

Equation 2. Area of silicon membrane for desired O_2 concentration.

$$A = \frac{RR_{O_2} \cdot m}{K_{O_2} \cdot PP_{O_2}} \qquad (3.2)$$

Where:

A = Area [m^2].

RR_{CO2} = Respiration rate [L/kg.day].

RR_{O2} = Respiration rate [L/kg.day].

m = Mass of the product stored [kg].

K_{CO2} = Permeability of the membrane for CO_2 [L/day.m^2.atm].

K_{O2} = Permeability of the membrane for O_2 [L/day.m^2.atm].

PP_{CO2} = Partial pressure difference across the membrane [atm].

PP_{O2} = Partial pressure difference across the membrane [atm].

The chamber operating with the silicon membrane system must guarantee good air tightness in order to build up and maintain the desired gas mixture.

Avocado fruit has a very high metabolic activity and its respiration rate is very high when compared to other fruits. It was intended to observe the behaviour of this commodity under the silicon membrane system and the viability of using such a system as a suitable storage system for avocado fruits. If a commodity as perishable as avocado fruit could be stored under this system other less perishable fruits with lower metabolic activity could also be stored using the same system.

3.3. Quality Evaluation.

Qualitative evaluations of agricultural products have been subject of interest of researchers for many years. Nevertheless, clear definition of what quality of agricultural products is, differs among different researchers. However, some basic factors commonly used to characterize quality are size, shape, colour, flavour, texture, firmness, defects and presence of foreign material. Many quality factors of agricultural products have been related to physical properties of the commodities; it is possible to develop destructive and non destructive methods to evaluate the quality based on physical properties.

The analysis of colour is frequently an important consideration when determining the efficacy of a variety of postharvest treatments. Consumers can easily be influenced by preconceived ideas of how a particular fruit or vegetable should appear, and marketers often attempt to enhance this physical property (McGuire, 1992).

The peel colour of ‘Hass’ Avocados is one of the factors by which consumers evaluate the freshness of the fruit. Peel colour of ‘Hass’ avocado is an important postharvest selection criteria. Depending on the variety, colour changes from bright green to dark-purple when ripe.

CHAPTER IV

STORAGE LIFE ENHANCEMENT OF 'HASS' AVOCADO FRUITS THROUGH MODIFIED ATMOSPHERE.

Abstract

In this study, a silicon membrane system was used for the storage of 'Hass' avocado fruits (*Persea americana* Mill. Var. Hass). The silicone membrane system is an efficient method for attaining and maintaining modified atmosphere (MA) in experimental storage chambers. The storage was performed in small sealed experimental chambers fitted with silicon membrane windows. The areas of the windows were calculated in order to achieve 3% oxygen assuming 30, 50, and 70% reduction of the respiration rate due to the effect of the modified atmosphere on the products' metabolic activity. Fruit stored at regular atmosphere (control) was kept under the same temperature (7°C) and relative humidity (90%) as those stored with silicon membrane system. The gas concentration in the chambers was analyzed using a gas chromatograph. The respiration rate was measured at storage (7°C) and ripening (15°C) temperatures. The effect of sulphur dioxide treatment on 'Hass' avocado fruit stored in the silicon membrane system was evaluated.

Fruit quality before storage, after storage and after ripening was also evaluated through physiological assessments. Fruit stored under the silicon membrane system remained in an excellent condition for 47 days. Following this period avocados ripened normally in a course of 4-10 days at 15°C and regular atmosphere. After ripening, the fruit did not show any apparent physiological deterioration or damage, neither development of undesirable organoleptic changes. The chambers with the small membrane area reached stable gas concentration in 6 days, the chambers with the large membrane area never reached steady gas concentration to the desired levels; while the chambers with medium membrane area

reached steady gas concentration in 15 days. Optimum results were obtained using small membrane area (28 cm^2 for a kilogram of stored avocado fruit) with the presence of sodium metabisulphate. This treatment has potential for commercial use after pilot scale studies.

4.1. Introduction.

The avocado has a very high rate of postharvest respiration and limited shelf life. Trade with distant markets requires production of high quality fruit and efficient transport so that it may arrive at the market in sound condition. Successful marketing must rely on decreasing the rate of ripening sufficiently to allow for shipping time (which is primarily by sea) and marketing. An inappropriate postharvest handling would be reflected in premature deterioration, which makes commercialization a challenge.

The response of avocado to temperature, particularly its susceptibility to low temperature, including preharvest conditions, varies among avocado cultivars and may be influenced by their geographic origin (Zauberman. et al., 1977). Eaks (1976) reported no ripening of avocados stored at 5°C or lower. No chilling injury occurred if fruit was stored at 10°C, but at 5°C chilling injury was found to increase with storage time longer than 2 weeks; it was also found that storage for longer than 5 days at 5.5°C caused abnormalities in subsequent respiratory patterns during ripening at 18°C.

Storage of avocado fruit, at temperatures lower than 10°C but higher than 0°C for relatively long periods of time results in chilling injury (Zauberman et al., 1985). In a study with 'Fuerte' avocados, Lee and Young (1984) reported development of chilling injury in fruits stored in air for more than 20 days at 6°C as indicated by grey discoloration of the mesocarp tissue; when fruits were stored with 100ppm ethylene tissue discoloration was severe at temperatures below 12°C, which implied that chilling sensitivity of avocado increased with ethylene. In the same study, 'Fuerte' avocado stored at 6°C remained firm even after 6 weeks, after which the experiment was terminated. These fruits began to show typical chilling injury after 3 to 4 weeks of storage but did not soften. At 9°C fruits

softened after 4 weeks; for fruits at the same temperature, but under ethylene treatment, chilling injury was severe after only 2 weeks of storage.

Hatton and Reeder (1972) reported that chilling injury was variable and could occur in avocados within two weeks at temperatures as high as 10°C. While they concluded that preharvest factors might contribute to susceptibility, postharvest factors such as gas components and ethylene contamination around storage units could have additional significant effects on chilling injury. Removal of ethylene from the storage atmosphere helps maintain avocado fruit quality.

Avocado fruit distribution to distant markets is a problem owing that the soft, ripe, and ready to consume fruit can not be transported or stored for more than a day or two (Peleg et al., 1990), while the consumer may soften the fruit as required. Thus, it is a common practice to store and ship pre-climacteric mature avocado, while the retailer, or consumer, may soften the fruit as required by storing it for two or three days at room temperatures.

Avocados are highly perishable commodities, with a maximum shelf life of four weeks after harvest (Oudit and Scott 1973; Wardlaw 1934). There is a need to prolong further the storage life of Avocados, both to extend the local marketing season and to facilitate export (Stother 1971). Special attention must be placed in its storage conditions.

There are series of methods that are available for storage that can be combined to reduce the ripening rate of perishable products. Nevertheless, when working with a product that has a very high metabolic activity and is highly susceptible to chilling injury like avocado fruit, more care must be taken when selecting the storage conditions. The published research indicates that the problem has not been fully solved.

Much of the literature on storage of subtropical and tropical fruits and vegetables is fragmentary and inconclusive. A storage temperature found to be optimal for a particular fruit in one country may be quite unsuitable for the same fruit grown elsewhere. So far the relevant storage literature is scanty; many of the storage temperatures cited refer to produce

obtained under temperate conditions. In the literature, some authors refer to avocados as a whole without specifying variety or state of maturity of the fruit, generalizing results as if all varieties behave similarly in terms of resistance or metabolic activity.

The use of modified and controlled atmospheres must be considered as a supplementary to cold storage. The success of this technique depends on the variety of the product, its physiological age, the composition of the atmosphere around the product, the temperature, and the duration of storage (Kader 1992). This explains the wide variability of results published for the same product.

The principal benefit that one can expect from controlled atmosphere storage is that the product so stored will maintain its freshness and eating quality for a longer period than it would if stored at the same temperature in air. The owner of such produce expects to market it at a time when both the quantity of available product is low, and the quality of the competing product, not stored in controlled atmosphere, is poor. With this marketing strategy one can expect to recover all the additional costs of the controlled atmosphere storage plus a reasonable profit.

Research into controlled atmosphere for avocado has been conducted sporadically for over 20 years (Ahmed and Barmore 1980). In the case of 'Hass' avocados, an oxygen concentration of 2% and carbon dioxide of 10%, at 5.5°C was found to provide the best extension of storage life and decrease in postharvest physiological disorders (Eksteen and Truter 1985). This is in agreement with Spalding and Reeder (1975) who were able to double storage life with an increase of fungal infection during ripening under similar conditions. Unfortunately the ratio of oxygen to carbon dioxide is important (Smock 1979). The careful control of the gas concentrations requires an air tight container with accurate control systems to be used between production and final marketing point. In general, such containers are not readily available at an economic price.

The membrane system creates a modified atmosphere due to the presence of a polymeric or elastomeric film which serves as a gas exchange barrier. There is a wide range

of film types with different permeability to different gases, water vapour, sealing, and mechanical resistance. The appropriate film to use depends on the metabolic activity of the commodity and the desired atmosphere for preserving its quality.

The silicon membrane system is an efficient method to achieve and maintain modified atmospheres in the storage of fruits and vegetables. The low permeability of the membrane to water vapour and its high permeability of ethylene provides important advantages over other modified atmospheric systems. It allows gases to diffuse at different rates according to their physical and chemical properties. With this system, the atmospheric composition is obtained through two simultaneous processes, membrane permeation and product respiration (Gariepy et al., 1986).

In this study, a silicon membrane system was proposed for the storage of 'Hass' avocado fruits; this system is relatively inexpensive and protects the produce from chilling injury, insects and microorganisms, reduces the respiration and transpiration rate of the commodity, and increases its storage life.

4.2. Objectives.

The objectives of this study were:

1. To determine whether the storage life of 'Hass' avocado fruits could be extended sufficiently by using the silicon membrane storage system and low temperatures to increase the commercialization time.
2. To determine the effects of sulphur dioxide treatment on stored 'Hass' avocado fruit in a silicon membrane storage system.

4.3. Materials and Methods.

Storage experiments were divided into three stages. In the first stage, the initial and final physical characteristics of the avocados, when left to ripen at 7 and 15°C, were evaluated. The physiological characteristics to be assessed for fruit quality included

moisture content, % loss due to microbial attack or chilling injury, peel colour, firmness, and % mass loss. In the second stage, the respiration rate of 'Hass' avocado was measured at 7 and 15°C in regular atmosphere. In the third stage, the respiration rate reduction under 3% oxygen and 3% carbon dioxide was estimated based on the respiration rate measured under regular atmosphere. The silicone membrane system was designed to maintain this gas mixture for optimum avocado storage. Three estimations were made for the changes in respiration rate which were likely to occur under this temperature and gas mixture conditions; a 30% decrease, a 50% decrease or a 70% decrease in the respiration rate. These estimations were used to predict the cross sectional area of the silicon membrane required. The effect of sulphur dioxide on stored avocados was also evaluated at this stage.

Sulphur dioxide was initially applied in the form of sodium metabisulphate pads. Moisture within the experimental chambers is absorbed and reacted with the sulphite to produce sulphur dioxide. The slow release of sulphur dioxide from the pads has proven to be very efficient; they are capable of releasing sulphur dioxide over a long period, enough to slow mould development, especially at low temperatures (Mustonen, 1992).

Fruit quality was evaluated by measuring physiological properties before storage, after storage and after ripening. These properties were used to quantify and qualify the quality and maturity stage of the stored product. Control and quantification of gas evolution inside the chambers was carried out during the entire storage period.

4.3.1. Storage facility.

All the ripening experiments were conducted in a walk in cold room of dimensions 4.5 x 3.0 m and 2.1 m high. The temperature of the cold room was set at 15°C and was maintained by a temperature control unit. The storage experiment was conducted in a control chamber of 1.4 m^2 set at 7°C and 90% relative humidity. The temperature in this chamber oscillated between 6.5 and 8°C for the whole storage period.

4.3.2. Experimental chambers.

All storage experiments were conducted in triplicates at 7°C and involved storage of 'Hass' avocado fruit in experimental chambers. The chambers were made from transverse sections of PVC pipe (turquoise), 32 cm in length and 11 cm in diameter. One end of the pipe was sealed with a solvent/polymer cement, with a PVC cap, on the other end a threaded PVC cap with its lid was glued to the pipe. The lid was modified in such a way that a circular plexiglass window, 6 mm thick, would allow for visual product inspection during storage. Two rubber sampling ports were built into the plexiglass window; these allowed sampling of the internal gases. Each chamber was of three litre capacity. Openings of calculated area were cut out of the PVC pipe (Figure 4.1).

The silicon membrane was fixed on the openings with the aid of silicon glue; the borders were reinforced with metalized tape in order to prevent escape or infiltration of gases. It consisted of a Tergal net (52-54 g/m^2) covered with a continuous coating of silicon rubber (≈ 90 μm). At 1 atm, its permeability to carbon dioxide is 1750 [L day^{-1} m^{-2}], to oxygen is 320 [L day^{-1} m^{-2}], and to ethylene is 700 [$L.day^{-1}.m^{-2}$], selectivity ratios were 5.5 and 2.5 for CO_2:O_2 and CO_2:C_2H_4, respectively (Gariepy et al., 1986). Procedures to calculate the membrane area required are based on the level of carbon dioxide or oxygen desired in the storage chamber. A chamber operating with the silicon membrane system must be relatively air tight in order to establish and maintain the desired gas mixture.

Neoprene gaskets were used at both ends of the PVC pipe to ensure air tightness. Chambers were tested for lack of leaks using a digital pressure indicator, DPI 601, with a resolution of 0.05% in the range 1 to 3500 kPa. The pressure inside the chamber was increased to 5kPa using an air pump and this pressure was maintained for 20 minutes. The chamber was considered air tight if no change in pressure was recorded over the 20 minute period.

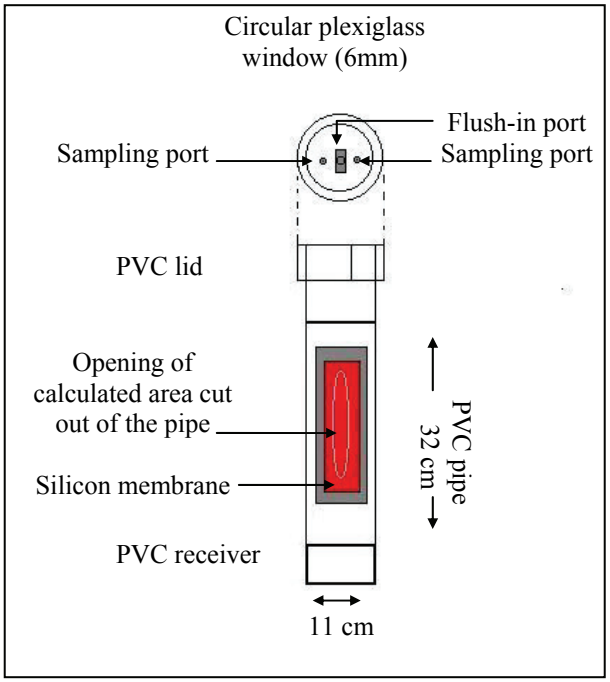


Figure 4.1: Experimental chambers.

4.3.3. 'Hass' avocado fruits.

One hundred and fifty mature but unripe preclimateric Avocados (*Persea americana* Mill. var Hass) were obtained through a local distributor, VIAU & FRERES Inc., (Dorion, Montreal, Quebec). The fruits were produced in Mexico and commercialized under the "Calavo" brand.

Calavo Growers of California is an enterprising association of 2,600 avocado growers; it handles 57% of the local avocado crop and 33% of the Florida crop, selling directly to the retail markets.

Calavo's 'Hass' avocados are gradually pre-cooled before packing. This allows the fruit to cool down over a 12 hour period from ambient temperature at the delivery time, to 4.4-7.2°C before packing the next morning. The fruits are classified and packed in a single layer in corrugated cardboard boxes, the boxes are sealed and refrigerated until shipping. In the ship the boxes are placed inside containers where temperature oscillates between 4.4 -

5.6°C. The fruit was in the ship for a week or two before arrival in Canada. Once in Canada, the boxes are transported by a truck (maintained at 5.6°C). Thus, the fruit took four days to reach Montreal. The Avocados are then received by VIAU & FRERES Inc. and stored at a temperature of 3.3°C for three more days, until acquired for our experiment. The fruits were of commercial grade and between 2-3 weeks from the harvest on arrival.

For each storage experiment, approximately 1 kg of avocados was weighed and placed in each chamber. Twenty typical fruits were taken and analyzed for the zero-time sample as soon as the fruits were received. The remainder were sorted into lots of 5 fruits which were put in storage chambers provided with silicon membranes and held at 7°C for seven weeks. Controls consisted of triplicates of original boxes containing one kilogram (5 avocados) in one layer of avocados under regular atmosphere.

4.3.4. Ripening of 'Hass' avocados at 15°C.

Ripening of a batch of twenty four green mature "Calavo" 'Hass' avocado fruits was conducted in a cold room set at 15°C. The ripening was monitored by measuring volume, mass, density, colour, firmness and water content. The initial and final physical characteristics of the avocado fruits when left to ripe were evaluated to determine softening time and quality before storage and after ripening. Twelve randomly picked fruits from the lot were used for the analysis.

4.3.5. Quantification and control of gas evolution within chambers during storage.

The respiratory gases, oxygen and carbon dioxide within each chamber were measured during storage using an SRI 8610A gas chromatograph with electronic integrator (SRI Instruments, California, USA) fitted with a thermal conductivity detector. The oven temperature was set at 45°C and the detector temperature set at 120°C. Helium was used as the carrier gas at 70psi. Data was recorded and analysed using a Peak Simple Chromatograph Data System Software for Windows, model # 203 (SRI Instruments,

California, USA). Two gas samples of 0.5 ml were taken from each storage chamber and injected into the gas chromatograph. Prior to analysis, the instrument was calibrated with a standard of known concentration, and the syringes used to collect the samples were made air tight with Teflon tape.

4.3.6. Measurement of respiration rate.

Twenty 'Hass' avocados were used to measure the respiration rate at 7 and 15°C (storage and ripening temperatures). The fruits were weighed and placed in three litre airtight containers for three hours. The respiratory gases, oxygen and carbon dioxide within each container were then measured. This procedure was replicated three times.

Avocados were left to ripe in open containers and the properties of 12 avocados from the lot were assessed after ripening. The respiration rate was calculated for each temperature using the average of the measurements. The Respiration rate was expressed as milligrams of carbon dioxide produced per kilogram of original fresh weight of stored product per hour (Ratti et al., 1998).

4.3.7. Modified atmosphere storage of avocados using the silicon membrane system.

The respiration rate at 3% oxygen and 3% carbon dioxide, was estimated to be 30% less, 50% less or 70% less than the respiration rate under regular atmosphere, the exact respiration reduction value is unknown for 'Hass' avocados stored under a modified atmosphere at these conditions of temperature, gas composition, and relative humidity. These estimations were used to calculate the area of silicon membrane required to achieve and maintain this gas composition using the equation proposed by Gariepy and Raghavan (1985). The membrane areas used were 64 cm^2, 46 cm^2 and 28 cm^2 for estimations of 30, 50 and 70% reduction in the respiration rate due to modified atmosphere. Chambers were flushed with a mixture of 90% nitrogen, 5% oxygen and 5% carbon dioxide for one minute, to allow the gas composition to approach 3% oxygen and 3% carbon dioxide. Gas

concentrations of 2 – 5% oxygen and 2.5 – 5.5 % carbon dioxide were accepted as suitable starting points. Carbon dioxide and oxygen evolution were determined by gas chromatographic measurement of the gas in the headspace of eighteen experimental chambers where five fruits (1 kg) were enclosed for the entire storage period. The respiration of the stored Avocado fruit and the properties of the membrane at these conditions of temperature and relative humidity maintained modified atmospheric conditions inside the chambers throughout the storage period.

The Avocados were held under the silicon membrane storage system for approximately seven weeks (47 days) at 7°C before being induced to ripening at 15°C and a regular atmosphere (RA). Quality parameters were assessed on the stored fruit before storage, after storage, and after ripening for all experiments undertaken, in order to determine the effectiveness of each treatment. Results for the beginning of the storage period, the end of the storage period and after ripening are the average of 20, 9 and 6 fruits, respectively, from each treatment.

4.3.8. Extension of storage life using sulphur dioxide.

Sulphur dioxide was tested for its ability to delay the incidence of fungal infection, thereby extending the shelf life of avocados at concentrations of one perforated 'sache' or pad of sodium metabisulphate per kilogram of stored avocado in combination with the silicon membrane system. The pads were placed at the bottom of the chambers; only nine chambers contained sodium metabisulphate pads, the remaining 9 were stored under the silicon membrane system without the pads.

The 2 x 3 cm pads contained 3 tablets (1.2 g) of sodium metabisulphate, pads or 'saches' produced in Tianjin, China by the National Engineers and Technologists Institute for freshness research. The principal component of this product is sodium metabisulphate ($Na_2S_2O_5$). Sodium metabisulphate releases sulphur dioxide (SO_2), which reacts with water vapour to form sulphuric acid (H_2SO_4) aerosol, which prevents enzymatic browning in

grapes. It is also antimicrobial, antioxidant, and traps the undesired acetaldehyde (detrimental effects of low oxygen levels include accumulation of ethanol and acetaldehyde to concentrations that can result in off flavours) and inhibits the growth of lactic acid and acetic acid bacteria.

4.3.9. Evaluation of Quality.

4.3.9.1. Peel colour.

The peel colour of 'Hass' avocados is one of the factors by which consumers evaluate the freshness of the fruit. Peel colour of 'Hass' avocado is an important postharvest selection criteria due to its dramatic peel colour change while ripening, from green to purple-black.

Peel colour was measured using a Minolta Chroma Meter, Model # CR-300b (Minolta Inc., Canada). Colour measurements were noted using L*, a* and b* scale. L* coordinate is a measurement for clarity (white-black and varies from 0 to 100). The a* scale varies from negative values for green to positive values for red. The b* scale varies from negative values for blue to positive values for yellow (McGuire 1992). Before each set of measurements, the Chroma Meter was calibrated using the manufacturers standard calibration plate with Y, x and y values set at 94.57, 0.31, and 0.32 respectively. Duplicate measurements were taken of four different positions on each avocado fruit and the average of each position used; three measurements were taken at the circumference of the avocados, and one was taken at the base.

4.3.9.2. Percent mass loss.

Cumulative mass loss during storage was calculated and expressed as percentage physiological mass loss on a wet basis.

4.3.9.3. Moisture content.

The moisture content of the pulp of avocado (wet basis) was determined by placing 20 grams of small pieces of the flesh on tared aluminium trays and left in a hot air oven at

75°C for two days. The samples were then re-weighed and the moisture content calculated. (Rahman et al., 1995).

4.3.9.4. Fruit firmness.

Firmness was determined by the required pressure to penetrate the avocado fruit through the peel using the Instron Universal Testing Equipment, model # 4502. Load cell of 50 kN and a flat-headed probe attachment (6 mm diameter) were used. Samples were individually compressed at a crosshead traveling speed of 20 mm/min. The Instron machine was connected to a computer for the data acquisition. The value recorded was the maximum penetration force required for the fruit to yield to the tip of the probe (Medlicott et al., 1990).

Before the storage period fifteen immature avocados from the whole batch were tested unpeeled, at three different positions on each avocado fruit; the average of each position taken. After the storage period three randomly picked fruits from each chamber were analyzed in the same way. The remaining fruit was left to ripen and analyzed after ripening.

4.3.9.5. Percent of damage.

For determining the damage to avocado fruits due to storage system, a method for calculating the percentage of loss was developed. After the storage period (three avocados after the storage period and 2 avocados after ripening per chamber), the fruit was cut in half and weighed without the seed, the apparent damaged parts of the fruit removed and weighed. The criterion was based on development of grey or dark brown discoloration of the mesocarp and blackening of the exocarp. The recorded values were expressed as a percentage of the total weight of the fruit without the seed. The mean value for each treatment was used for analysis.

4.3.10. Experimental design and statistical analysis.

A completely randomized design (CRD) was used with seven treatments, before storage, after storage and after ripening. The treatments used were as follows: regular atmospheric storage (control), modified atmosphere storage with 64, 46, and 28 cm^2 silicon membrane, modified atmosphere storage with 64, 46, and 28 cm^2 silicon membrane and sulphur dioxide treatment. Each of these treatments was replicated three times.

Analysis of variance (ANOVA) and pairwise comparisons of means using the Fisher's LSD test were performed with the statistical analysis software system (XLSTATS V.6.0). Treatments were compared at $P < 0.05$ (Least Square Difference LSD). Data obtained from fruits was subjected to ANOVA and the means were separated using the Fisher's LSD multiple comparison test.

4.4. Results and Discussion.

This section contains results of experiments conducted to investigate the storability of preclimateric avocados using the silicon membrane system and a combination of the silicon membrane system with SO_2 as a chemical inhibitor of decay.

4.4.1. Effect of membrane area on the avocado quality/physical properties.

4.4.1.1. Storage.

Physical properties of 'Hass' avocado fruit after 47 days of storage under MA with and without sulphur dioxide treatment are shown in Table 4.1. The physical properties after ripening of 'Hass' avocado fruit stored for 47 days under MA with and without sulphur dioxide treatment are shown in Table 4.2. Neither the mass nor moisture content of avocados changed among treatments during the storage period. This is attributable to the high relative humidity inside the chambers. Table 4.2 also compares the ripening parameters of the control group at RA after 18 days with those stored for 47 days under MA. MA storage after 47 days and ripening is equivalent to RA storage for 18 days.

Table 4.1: Physical properties of 'Hass' avocado fruit after 47 days of storage under MA with and without sulphur dioxide treatment.

	Control						
	Day 0				**+ SO_2**	**+ SO_2**	**+ SO_2**
Parameters	**RA**	**28 cm^2**	**46 cm^2**	**64 cm^2**	**28 cm^2**	**46 cm^2**	**64 cm^2**
Mass [kg]	0.17a	0.16a	0.17a	0.16a	0.16a	0.16a	0.16a
% Moisture							
(wb)	77a	78a	80a	82a	82a	77a	80a
% Damage	0a	11ab	7ab	50c	1[a]	35abc	45bc
Firmness							
(N)	60.57a	3.99b	3.55b	2.86b	5.05b	2.94b	3.16b
Colour							
L*	55.56a	29.57bc	27.93bd	25.51d	32.62c	30.07bc	31.09bc
a*	-12.20a	-3.78b	1.68c	0.86cd	-3.59b	-0.82e	0.34de
b*	18.66a	9.22b	4.92c	5.14c	11.24d	8.15be	7.18e

All values are expressed as the mean of $n \geq 3$
Means with the same letters in the same row are not significantly different at 95% confidence interval.

Table 4.2: Physical properties after ripening of 'Hass' avocado fruit stored for 47 days under MA with and without sulphur dioxide treatment.

Parameters	28 cm^2	46 cm^2	64 cm^2	+ SO_2 28 cm^2	+ SO_2 46 cm^2	+ SO_2 64 cm^2	Day 18th RA
Mass [kg]	0.16ab	0.16ab	0.16ab	0.16ab	0.17b	0.16ab	0.15a
% Moisture (wb)	78a	82ab	83b	83b	81ab	80ab	78a
% Damage	16ab	29abc	47bc	35abc	62c	58c	0a
Firmness [N]	3.49a	2.86b	2.61b	4.44c	3.19ab	2.97ab	3.16ab
Colour							
L*	33.48a	31.17a	32.55a	32.13a	34.00a	32.81a	48.96b
a*	0.79ab	1.47b	1.2b	-0.57c	0.42abc	0.96ab	-0.38ac
b*	6.63ab	3.64c	3.81c	7.98b	7.05ab	6.06a	10.73d

All values are expressed as the mean of $n \geq 3$
Means with the same letters in the same row are not significantly different at 95% confidence interval.

4.4.1.2. MA storage conditions.

The different size of silicon membrane area calculated and used for the storage of 1kg of 'Hass' avocado kept the product under different modified atmospheres (MA). Figure 4.2 shows the progression of CO_2 and O_2 concentrations as a function of time for the fruits stored in chambers fitted with a 28 cm^2 of silicon membrane. From the graph it can be seen that during the first few days of storage the levels of carbon dioxide dropped and the levels of oxygen increased within the chambers until reaching a steady-state value of carbon dioxide of around 2 – 2.5%. Once these levels of carbon dioxide were achieved the gas evolution inside the chambers followed the trend characteristic of the silicone membrane system described by Gariepy et al., (1984).

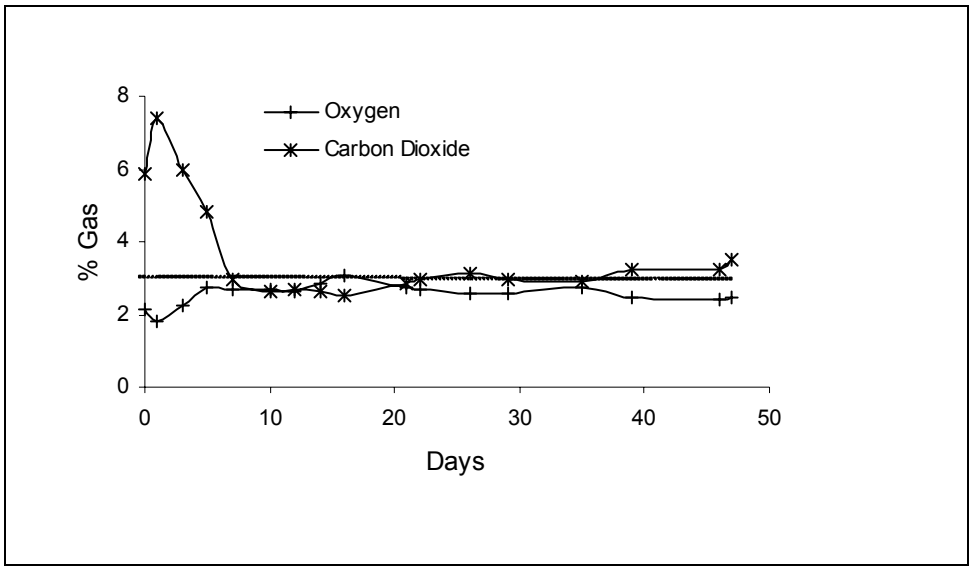


Figure 4.2: Gas evolution in experimental chambers fitted with small size membrane (28 cm^2) during the storage period of 'Hass' avocado fruits. The horizontal line represent the expected gas concentration of 3% for which the area was calculated.

The fruit stored under the membrane system designed for 30 and 50% reductions in respiration rate, followed a similar trend; the size of the membrane had a direct effect on the amount of oxygen accumulating and carbon dioxide being depleted from the chambers during the first few days of storage. Within the chambers fitted with large membrane areas, the levels of oxygen were higher and the levels of carbon dioxide were lower than for chambers fitted with smaller membrane areas. There are two possible explanations for this behaviour: The sensitivity of avocado fruit to high concentrations of carbon dioxide and the significant reduction of respiration rate due to the effect of modified atmosphere and low temperature conditions. The silicon membrane system had been used before with fruits and vegetables with lower metabolic activity. One must remember that the atmospheric composition around the stored product under this system depends on various factors, the metabolic activity of the commodity, the permeability of the membrane to the gases, and the size of the membrane.

The silicon membrane system maintained the modified atmosphere conditions within the experimental chambers at 2 – 5 % carbon dioxide and 3 – 7 % oxygen, in the chambers designed for 30% respiration rate reduction, at 2 – 4 % carbon dioxide and 3 – 6 % oxygen in the chambers designed for 50% respiration rate reduction, and at 2 – 7% carbon dioxide and 2 – 3 % oxygen in the chambers designed for 70% respiration rate reduction of the stored product. When combined with sulphur dioxide treatment the silicon membrane system kept atmosphere within the experimental chambers during the storage period at 2 – 4 % carbon dioxide and 3 – 8 % oxygen in chambers designed for 30% respiration rate reduction, of 2 – 4 % carbon dioxide, 3 – 5 % oxygen in chambers designed for 50% respiration rate reduction, and 3 - 5 % carbon dioxide and 2 – 3 % of oxygen in chambers designed for 70% respiration rate reduction of the stored product.

The speed at which the optimum gas content was achieved had a substantial effect on the quality of the avocado fruit after storage. Chambers with a small membrane area (28 cm^2) stabilized the gas concentration at about 2.5% oxygen and 3.45% carbon dioxide in about six days and the quality of these fruits was better in terms of colour retention and overall appearance when compared with the fruits stored under different MA conditions; the gas levels for this treatment were very close to the designed values of 3% oxygen and 3% carbon dioxide. Chambers with medium size membrane area (46 cm^2) stabilized gas concentrations at about 4% oxygen and 3% carbon dioxide over fifteen days, while chambers with a large membrane area (64 cm^2) stabilized the gas composition to levels of 4.34% oxygen and 2.77% carbon dioxide in about twenty four days.

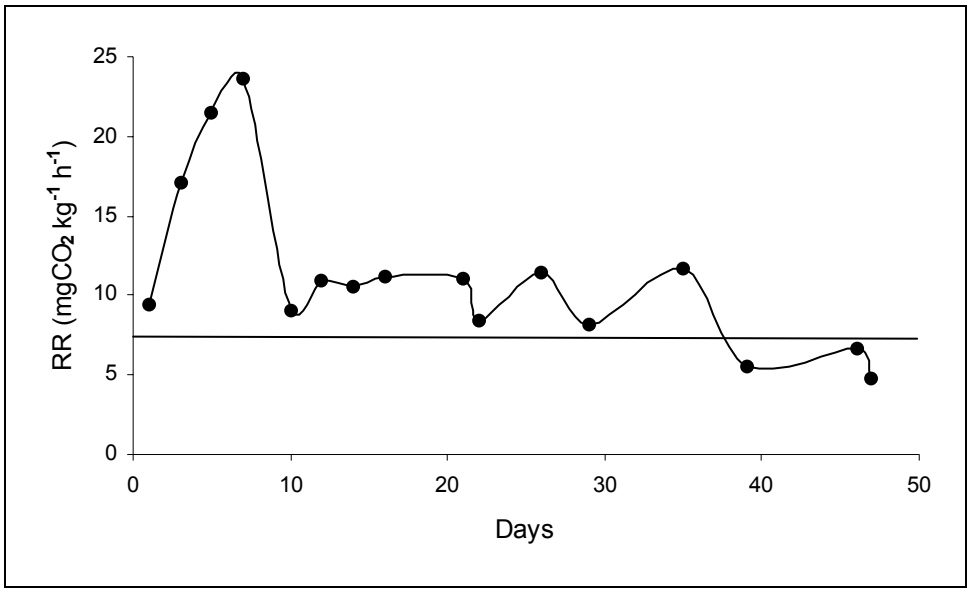
4.4.1.3. Respiration Rate.

The stored avocado, as a typical climacteric fruit, displayed a characteristic peak of respiratory activity, termed respiratory climacteric. The magnitude of the peak and date of occurrence varied among treatments. The climacteric peaks for the fruit stored under MA in chambers fitted with large, medium and small silicon membrane areas were observed to

have, on the first, second and seventh days of storage, values of 42 ± 4 mg CO_2 kg^{-1} h^{-1}, 38 ± 2 mg CO_2 kg^{-1} h^{-1} and 28 ± 5 mg CO_2 kg^{-1} h^{-1}, respectively.

It is interesting to note that the respiration rate (RR) of fruit stored in chambers fitted with medium (46 cm^2) and large (64 cm^2) silicon membrane area windows was higher and the climacteric peak occurred sooner than those stored in chambers fitted with small (28 cm^2) silicon membrane windows. The fruit stored in these chambers also tended to ripen more rapidly. It seems that the RR of avocado fruit changes significantly with varying gas compositions at lower temperatures.

The average respiration rate of fruit stored under the large size area was 16 ± 0.3 mg CO_2 kg^{-1} h^{-1}, this RR was achieved on the fifth day of storage and remained constant for twenty six days, time after which a decreasing tendency was observed. For the fruits stored under the medium size area, the average respiration rate was 12 ± 0.35 mg CO_2 kg^{-1} h^{-1} after the fifth day of storage and remained constant for about thirty three days, decreasing slightly after this period of time. The respiration rate was calculated and plotted as a function of time (Figure 4.3.) for the silicon membrane system (28 cm^2 of silicon membrane area and 1kg of fruit) during the 47 days of our study. The average respiration rate of the fruit stored under the small membrane area was 9 ± 0.45 mg CO_2 kg^{-1} h^{-1} and it was achieved on the tenth day of storage and remained constant for the entire storage period. The reported values of RR under MA were close to 70, 50 and 30% of that in RA at the same temperature.



a. **Figure 4.3:** Temporal variation of respiration rate of 'Hass' avocado fruit
b. stored in experimental chambers fitted with a small size membrane
c. (28 cm^2). The horizontal line indicates the expected respiration
rate of 7.4 ($mgCO_2$ kg^{-1} h^{-1}) at these conditions of MA.

Fruit stored in the chambers with small membrane areas had the lowest respiration rate throughout the storage period when compared to those stored in chambers fitted with other sizes of silicon membranes. The reduced temperature lowers the respiration rate, along with the reduced oxygen. Carbon dioxide has a limited direct inhibitory effect on the respiration rate. Optimal concentrations of carbon dioxide in avocado storage retard fruit ripening.

4.4.1.4. Peel Colour (L*, a* and b*).

When treated with sulphur dioxide, the size of the membrane did not have a significant effect on the clarity of the fruit (coordinate L*), but for those untreated, fruit stored in chambers equipped with 64 cm^2 silicon membranes were more opaque ($P < 0.05$) than those stored in chambers with smaller membrane sizes.

Unripe avocados have very low a* values. The size of the membrane had an effect on the retention of the green colour, the chambers with small membranes kept the stored fruit greener ($P < 0.05$) than those stored in chambers fitted with larger membrane sizes; sulphur dioxide treatment kept the fruit greener in chambers with medium size silicon membrane windows than those untreated in chambers with the same size window.

Unripe avocados have high positive values for b*. The size of the membrane and the presence of sulphur dioxide have an effect ($P < 0.05$) on colour retention; ripe purple avocados have an intermediate value for the b* coordinate. Non-treated fruit stored in chambers with medium and large membrane areas turned purple after the storage period but remained relatively firm. Firmness of the avocados was not affected neither by the size of the membrane nor by sulphur dioxide treatment after the storage period.

4.4.1.5. % Damage.

Fruit in the control treatment was of very poor quality after long term cold storage. Fruit held at 7°C in regular atmosphere were seriously decayed, very soft and senesced. In contrast, avocados stored under the silicon membrane system were rated marketable after 47 days of storage. They were firmer, had better appearance, aroma (the controls smelled fermented) and flavour, reduced incidence of chilling injury, and inhibition of fungal growth.

There was a direct relationship between oxygen availability and % damage. Fruit stored in chambers with large membrane areas retained higher levels of oxygen throughout the storage period than those stored in chambers with smaller membrane areas, resulting in more damage (stem rot and chilling injury) after storage. Low levels of oxygen and high levels of carbon dioxide reduced the incidence of chilling injury; oxygen requirements for microbial activity were not high enough and the high levels of carbon dioxide served as an antimicrobial agent. Chambers designed for 70% respiration rate reduction kept the gas composition closer to the desired levels of 3% oxygen and 3% carbon dioxide for a longer

period of time and the overall quality of the stored fruit under these MA conditions was slightly better.

4.4.1.6. Ripening.

After forty seven days of storage at 7°C under the silicon membrane system, 'Hass' avocado fruit resumed normal climacteric and softening upon transfer to 15°C conditions. The fruit ripened normally over a course of 4-10 days without the application of exogenous ethylene; flavour was good and there was no evidence of internal breakdown. On the other hand, fruits maintained at 7°C, for an additional 10 days slowly ripened and, on cutting, showed a slight internal discolouration indicative of abnormal ripening caused by the longer exposure to lower temperature.

The untreated fruit stored in chambers with large and medium size membranes did not develop proper purple colouration; it was lighter than normal, but softened properly over a six day period. The fruits treated with sulphur dioxide were firmer and developed a nice purple colouration after six days of ripening, especially the ones stored in chambers with the small membrane areas; these fruits were firmer ($P < 0.05$), requiring an additional four day period for proper softening.

The silicon membrane system possesses a suitable partial permeability to carbon dioxide and oxygen that can be used in conjunction with moderate refrigeration to handle 'Hass' avocado fruits. It is an efficient method to achieve and maintain modified atmospheres in the refrigerated storage of these fruits.

The low permeability of the silicon membrane to water vapour and the high permeability of it to ethylene provides important advantages over other systems. The storage life of 'Hass' avocado fruit was enhanced by seven weeks.

It is important to research the basic concepts of the effects of modified and controlled atmospheres on tropical and subtropical horticultural products in order to increment and improve the use of this technology.

The oxygen reduction in the air tight experimental chambers could have been left to act naturally; however a mixture of 90% nitrogen, 5% oxygen and carbon dioxide was used to establish a rapid initial drop. There have been questions regarding how rapidly the oxygen should be reduced to the proper level in controlled atmosphere rooms. In this study it was found that the longer the delay in establishing the desired 3% oxygen, the poorer the avocado fruit condition.

An inconvenience of the membrane system is that the containers have to be opened before ripening at ambient temperature as oxygen deficiencies promote the development of physiological disorders. The necessity to open the containers constitutes a severe commercial disadvantage.

Future development of more suitable materials could make the technique more attractive, for example developing materials that change permeability at the same rate as the temperature changes, or developing automatic systems that modify the membrane area with product, mass, and/or temperature changes.

4.5. Conclusions.

From this study we can conclude that the best treatment for storage of 1kg of 'Hass' avocado fruit was the silicon membrane system (28 cm^2) and low temperature (7°C) with sulphur dioxide. Sulphur dioxide seems to delay colour changes and softening of the fruit; further research should be done to determine the optimum amount required per kg of product to enhance avocado storage.

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CONNECTING TEXT.

Although an increase in the postharvest life of the avocado fruit was achieved through modified atmosphere storage in the previous chapter, the best storage place for an avocado fruit is the avocado tree. A better understanding of the phenomenon taking place while an avocado fruit remains attached to the tree is required.

Vacuum infiltration technique has proven to be an efficient method to infiltrate liquids through intact-fresh produce. In order to simulate the effects of growth plant regulators transferred from the plant to the fruit while being attached to it and to understand their effect in preventing ripening, vacuum infiltration technique was chosen among other methods to supply harvested avocado fruit the phytohormones (plant hormones) supplied by the parent tree and evaluate their effect in postharvest life of "Hass" avocado fruit.

The whole benefits of pressure infiltration of chemicals through the skin of whole fresh fruits and vegetables need to be demonstrated, many practical difficulties are envisaged for identifying effective infiltration techniques, particularly with respect to the thickness of the skin.

Here an experimental "Shock" treatment (high vacuum levels and shorter times of exposition) was proposed to infiltrate whole and intact "Hass" avocado fruits with the phytohormones in order to study the effects of growth regulators in postharvest treatments and to determine whether it is possible to simulate the conditions that prevent avocado fruits from ripening while attached to the tree.

CHAPTER V

PHYTOHORMONES IN POSTHARVEST LIFE OF ´HASS´AVOCADO FRUIT.

Abstract

Observations were made to determine the effects of several plant regulators, 2,4-dichlorophenoxyacetic acid, gibberellic acid, and 6-benzylamino purine on the respiration pattern, ethylene production, and the number of days to ripen of avocado fruits (*Persea americana* Mill.). These substances were vacuum infiltrated to insure good penetration and distribution. The results indicated that these hormones inhibited the rate of degreening and softening; although at comparable concentrations 2,4-dichlorophenoxyacetic acid was a more effective inhibitor of ripening. The metabolic activity of the treated fruit, as judged with the respiration rate, was diminished; 6-benzylamino purine was more effective than the other hormones in reducing respiration rate throughout the ripening period. Stimulation of ethylene synthesis by hormone application was observed; however, enhanced ethylene evolution could not overcome the inhibition of degreening, softening, and the respiratory activity by the phytohormones. The results reinforce several previous observations with other fruits that auxins, gibberellins, and cytokinins may largely constitute 'resistance to ripening' and may be responsible for the lack of ripening shown by unpicked fruits.

The results of this work will be used in using appropriate strategy for postharvest handling steps of avocados.

5.1. Introduction.

The effects of ethylene on the postharvest development of fruits and vegetables have been extensively studied. However there are many plant hormones whose potential utility in postharvest technology needs to be explored. Plant hormones rarely, if ever, function alone. Even in cases in which a response can be evoked by application of a single hormone, the tissue may contain additional endogenous hormones that contribute to the response. Senescence is controlled by hormones; growth stimulators (auxins, gibberellins and cytokinins) delay it, and growth inhibitors (ethylene and abscisic acid) speed up the senescence process. Ripening in fruit may be retarded by applying inhibitors of respiration and ethylene production at postharvest; however, the ripening process, once started, cannot be reversed.

The avocado differs from most other fruits in that ripening does not normally take place on the tree, but only after picking (Burg and Burg, 1962).

The best storage place for tree-ripe avocado prior to harvesting is on the tree. However, due to winds and weak stems, some fruits fall off the tree and may lay on the ground for several days before they are picked up and these are usually shipped to the packinghouse together with the freshly picked avocados (Peleg et al., 1990).

Fruits of many species ripen faster when detached from the tree, and slower or not at all if left on the tree. The inability of these fruits, notably some varieties of avocado (Gazit and Blumenfeld, 1972), to ripen on the tree, coupled with the lack of response by freshly harvested fruits to ethylene treatment, have prompted several workers to propose the existence of a ripening inhibitor in attached fruits (Dilley, 1969). Lieberman (1979) concluded that some anti-ethylene factor must be removed, or tissue must acquire increased sensitivity to ethylene before a reaction is triggered. This inhibitor is presumed to be formed in leaves of the parent tree and translocated to the fruit while still attached (Vendrell and Palomer, 1997). Following harvest, the inhibitor is initiated during the preclimacteric period and ripening is initiated by endogenously produced ethylene.

Moreover, 'Hass' fruit did not respond to ethylene treatments given immediately after harvest. Gazit and Blumenfeld (1970) attributed this behaviour to an endogenous factor inhibiting ethylene action. Adato and Gazit (1974) explained these results by the assumption that the factors which prevent avocado ripening on the tree, continue to exert their influence for a limited period after harvest and inhibit the ripening response in the freshly picked fruit. It is therefore conceivable that the resistance to ripening, which is displayed, for example, by fruit at early stages of maturity or attached avocado fruit, reflects the action of inhibitory levels of senescence retarding hormones in the fruit tissue (Frenkel and Dyck, 1973).

Cytokinins, auxins and plant growth retardants will generally delay senescence in vegetables. Cytokinins have been shown to be effective in delaying senescence in cabbage, lettuce, cauliflower, asparagus, broccoli, celery, brussel sprouts and other vegetables such as endive, escarole, mustard greens, spinach, radish, carrot tops, parsley and green onion. Isopentenyl adenosine (IPA) suppressed ethylene production in pre- and postclimacteric tissue of tomatoes and avocados (Lieberman et al., 1977). If ethylene production in tissue slices of ripening fruits is an index of aging, then IPA would appear to retard aging in ripening fruit, just as other cytokinins appear to retard aging in senescent leaf tissue.

The effects of exogenously applied auxin or auxin analogs on harvested fruit have been studied (Frenkel and Dyck, 1973). Some discrepancies can be observed from these results. For example, auxins have been reported to be effective in delaying senescence of cauliflower, broccoli and Brussels sprouts (Weaver, 1972); other authors demonstrated inhibitory properties of auxins in the delay of ripening processes in bananas (Vendrell, 1969) and pears (Frenkel and Dyck, 1973) following treatments with either 2,4-dichlorophenoxy-acetic acid (2,4-D) or indoleacetic acid (IAA). The postharvest application of auxins prevented the climacteric rise in respiration, but stimulated ethylene synthesis. Despite the presence of elevated ethylene levels, the inhibitory auxin effect on

Bartlett pears and bananas was dominant. It was proposed that endogenous auxins in fruit represent a resistance factor in ripening and must be inactivated before ripening can occur.

Maxie and Crane (1967) provided evidence that an auxin analog hastened maturity of fig fruit indirectly through stimulation of ethylene synthesis. Previous studies by these and other investigators have addressed auxin stimulated ripening of a wide range of fruits; in avocado fruit (Adato and Gazit, 1976) supra optimal concentrations of indole-3-aceti acid advanced ripening by about eight days in comparison to the control.

The dual effect of auxin could therefore lead to ambiguous results if care is not taken to insure the penetration of the auxin solutions into the fruit. Surface application of auxins to intact fruit results in an enhanced ethylene levels but only a few peripheral cell layers are inhibited by the auxin (Frenkel and Dyck, 1973). Under such experimental conditions the auxin treatment results in an ethylene effect and will actually cause an acceleration of ripening. Penetration of auxin solutions into the fruit as a result of vacuum infiltration assured that the inhibitory effect of auxin became predominant despite of elevated ethylene levels.

Gibberellin delays fruit maturation and ripening (Dilley, 1969). Dostal and Leopold (1967) found that postharvest application of gibberellic acid to mature green tomatoes or tissue slices delayed ripening and were insensitive to ethylene applications. Gibberellin can delay the progress of some components of fruit ripening, preventing some of the changes trigged by ethylene. Regreening of citrus fruit suggested the possibility of bringing the fruit to a less mature stage by the application of gibberellin (Goldschmidt, 2001).

Russo et al., (1968) reported that gibberellic acid applied to mature green bananas significantly delayed ripening. Abdel-Gawad and Romani (1967) observed that a postharvest application of gibberellic acid retarded ripening of apricot fruits measured by reduction in respiration rate and rate of flesh softening.

The manipulation of fruit ripening is of major economic importance. Plant growth substances such as auxins, cytokinins and gibberellins have been shown to reduce or delay

various aspects of ripening. While these plant growth substances play other roles, they each share the ability to delay senescence, thereby reducing the sensitivity of the fruit to ethylene (Abeles et al., 1992). The seed tissues in the immature avocado fruit have a very high content of auxins, gibberellins and cytokinins, which enable the seed to be a strong metabolic sink (Adato and Gazit, 1976); as the fruit becomes mature, the seed coat shrivels and dies and the seed no longer plays the role of a sink.

The avocado has a high rate of postharvest respiration and a limited shelf life. Exporters must rely on decreasing the rate of ripening sufficiently to allow for shipping time and marketing. Because attached avocado fruit resists ripening for long periods, we decided to test the effects of hormones on the ripening of whole, intact, detached avocado fruits. An understanding of biological activity of growth regulators, both natural and synthetic, in delaying or hastening fruit ripening and senescence would be of great value in developing and incorporating new technologies to preserve horticultural commodities.

5.2. Objective.

The objective of this study was to determine the effects of 2,4-dichlorophenoxyacetic acid, gibberellic acid, and 6-benzylamino purine postharvest treatment on the ripening pattern of 'Hass' avocado fruit.

5.3. Materials and Methods.

One hundred and eighty mature but unripe postclimacteric Avocado fruits (*Persea americana* Mill) of avocado cultivar 'Hass' were obtained through a local distributor (IMEX Inc., Montreal, Quebec). The fruits were grown in Mexico and commercialized under the "Fat Cats" brand. Fruits were sampled at the beginning of the experiment and again after ripening at room temperature. The avocados were left for 24 h at room temperature prior to hormone application.

Solutions containing 2,4-dichlorophenoxyacetic acid (2,4-D), gibberellic acid (GA_3) or 6-benzylamino purine (6-BA) were applied to the fruit according to the procedure of Frenkel et al., (1969) with slight modifications. Infiltration of the solutions was regulated by the vacuum setting at which the fruits were equilibrated prior to infiltration (Figures 5.1., 5.2., and 5.3.). For practical reasons, it was preferable to use unpunctured-whole fruits. A flash treatment was developed which assured intake levels of solution comparable to similar studies with perforated fruits. It was observed that the time of permanency under the vacuum regime had an inverse relationship with the volume infiltrated, being more efficient for shorter times. Fruits were submerged for 1 min at 100 mmHg, followed by infiltration for 35 minutes at atmospheric pressure to minimize the development of anaerobic conditions. Solutions used were of auxin, gibberellin or cytokinin, all at 10^{-3} M, were used. The hormone concentration was similar to those used in other studies (Dostal and Leopold, 1967; Vendrell, 1969; Gazit and Blumenfeld, 1970; Lieberman, 1979). Infiltration procedures employed 0.3 M D-mannitol (Frenkel et al., 1969), a sugar alcohol, as a carrier solution for administering protein and nucleic acid precursors.

In accordance with Frenkel and Dyck (1973), the volume of treating solution which could be infiltrated into avocado fruits followed a relationship as shown in Figures 5.1. and 5.2. In a preliminary test the penetration of dip was studied using methylene blue as a dye solution. Uniform distribution throughout the tissue was observed in its vascular system. Infiltrated fruit was left to ripen at room temperature and compared with water-infiltrated, D-mannitol-infiltrated, evacuated fruit as well as uninfiltrated fruit. Samples were taken at intervals for measurement of the ripening process and the evolution of ethylene and carbon dioxide.

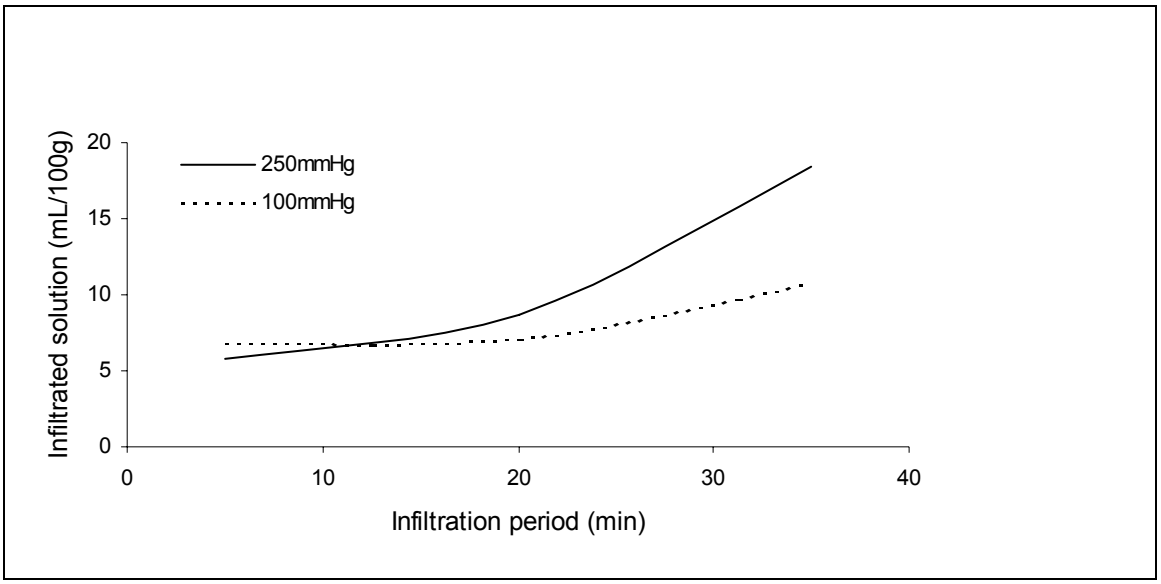

Figure 5.1: The rate of uptake of an ambient solution by submerged 'Hass' avocado fruit at atmospheric pressure after evacuation of the intercellular atmosphere to 250mmHg and 100mmHg.

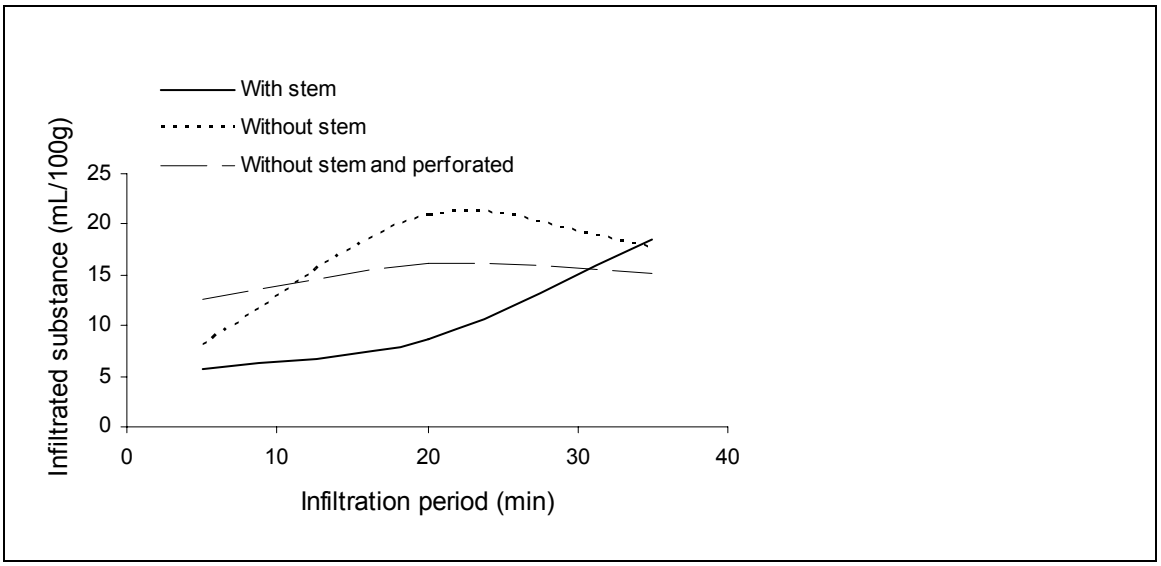

Figure 5.2: The rate of uptake of an ambient solution by submerged 'Hass' avocado fruit with stem, without stem, and perforated without stem at atmospheric pressure after evacuation of the intercellular atmosphere to 250mmHg.

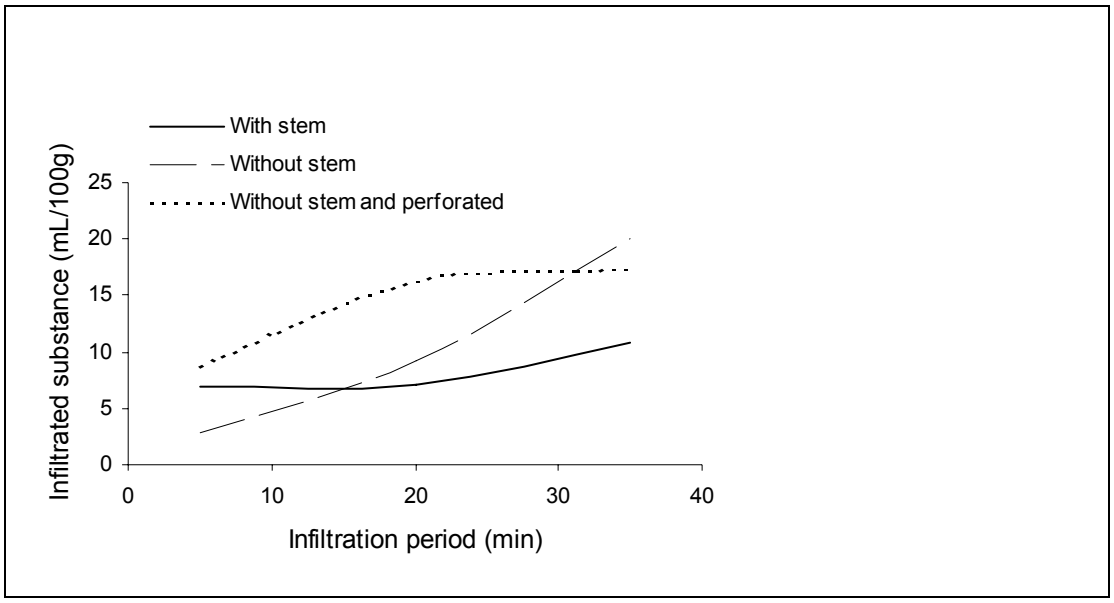

Figure 5.3: The rate of uptake of an ambient solution by submerged 'Hass' avocado fruit with stem, without stem, and perforated without stem at atmospheric pressure after evacuation of the intercellular atmosphere to 100mmHg.

Softening and peel colour were used as indicators of ripening. Softening was measured on individual fruits, before ripening and after ripening, with Instron Universal Testing Equipment, model # 4502. The test was conducted on unpeeled avocado fruits. A load cell of 50 kN and a round-headed probe attachment (8 mm diameter) were used. Samples were individually compressed at a crosshead traveling speed of 20 mm/min. The Instron was connected to a computer for the data acquisition. Peel colour development of each fruit was tested daily, just before determination of its carbon dioxide and ethylene production, using a Minolta Chroma Meter, Model # CR-300b (Minolta Inc., Canada). Colour measurements were registered using Hunters L*, a* and b* scale. The L* coordinate is a measure of clarity (white-black and varies form the point L* = 0, to the point L* = 100). The a* scale varies from negative values for green to positive values for red. The b* scale varies from negative values for blue to positive values for yellow (McGuire, 1992). Before each set of measurements, the Chroma Meter was calibrated

using the manufacturers standard calibration plate with Y, x and y values set at 94.57, 0.31, and 0.32 respectively. Duplicate measurements were taken at four different positions on each avocado fruit and the average of these positions was used; three measurements were taken at the widest circumference of the avocados, and one was taken at the base. Five fruits were used for each of the above determinations. All the experiments were replicated three times.

Carbon dioxide evolution was measured at 24 h intervals for the length of the experiment. Two randomly picked fruits from each lot were weighed and placed in a 1 L air tight container for one hour at room temperature. The respiratory gases, oxygen and carbon dioxide within each jar were measured using a SRI 8610A gas chromatograph with an electronic integrator (SRI Instruments, California, USA) fitted with a thermal conductivity detector. The oven temperature was set at 45°C and the detector temperature set at 120°C. Helium was used as the carrier gas, at 70 psi. Data was recorded and analyzed using a Peak Simple Chromatography Data System Software for Windows, model # 203 (SRI Instruments, California, USA). Two gas samples of 0.25 mL were taken from each container and injected into the gas chromatograph (GC). Prior to analysis, the instrument was calibrated with a standard of know concentration, and the syringes used to collect the samples were made air tight with Teflon tape.

Ethylene production was assessed using a Hewlett Packard GC (model no. 5890A) coupled to a flame ionization detector. The GC was equipped with a glass column packed with alumina F-1 (80/100 mesh). The carrier gas was helium at 25 $mL.min^{-1}$ with column head pressure at 100 kPa. Measurements were taken on the fourth and fifth day of the ripening period, following the same procedure as of carbon dioxide evolution. Data was recorded and analyzed using the Peak Simple Chromatography Data System. This instrument was calibrated using standard ethylene. Gas concentrations were obtained directly from the integrator's reports. The column, injector and detector temperatures were

45, 60, and 220 ºC respectively. The mean values of these two day measurements were recorded and used for analysis.

There seem to be a possibility that enclosing the fruit in an air tight container for a short period of time during ripening, might affect the fruit ripening rate. Hence, six fruits from each treatment were sealed for 30 min either every day or twice a day on the fourth and fifth day of the ripening period, but no significant effect on ripening rate or ethylene production was found.

A complete randomized design (CRD) was used with seven treatments, during and after ripening. Each of these treatments was replicated three times.

Analysis of variance (ANOVA) and the pairwise comparison of means using the Fisher's LSD test were performed by statistical analysis software system (XLSTATS V.6.0). Treatments were compared at $P < 0.05$ (Least Square Difference - LSD). Data obtained from fruits were subjected to ANOVA and the means were separated using the Fisher's LSD multiple comparison test.

5.4. Results and Discussion.

The effects of auxin, gibberellin and cytokinin on ripening and on carbon dioxide evolution are shown in Figures 5.4. and 5.5.

The current study supports previous suggestions that auxins function as a resistance factor in fruit ripening (Frenkel and Dyck, 1973). The stage of fruit development at the time of auxin application apparently has a pronounced effect upon the response (Dilley, 1969); the flash treatment developed here should be tried in less mature avocado fruits. If the growth hormones (auxins, gibberellins and cytokinins) can suppress aging, they will likely be most effective in the preclimacteric stage, before reactions associated with aging are fully set in motion; the ripening process, once started, cannot be reversed.

The effects of the different treatments on carbon dioxide evolution are shown in Figure 5.5. Although the changes in respiration in relation to auxin treatment are not as

clear cut as degreening or softening, it is evident that overall metabolic activity, as judged with the respiration rate, was diminished, but ethylene synthesis was stimulated. The respiration rate of fruits treated with the hormones followed the same pattern, although 6-BA was more effective in reducing respiration rate throughout the ripening period.

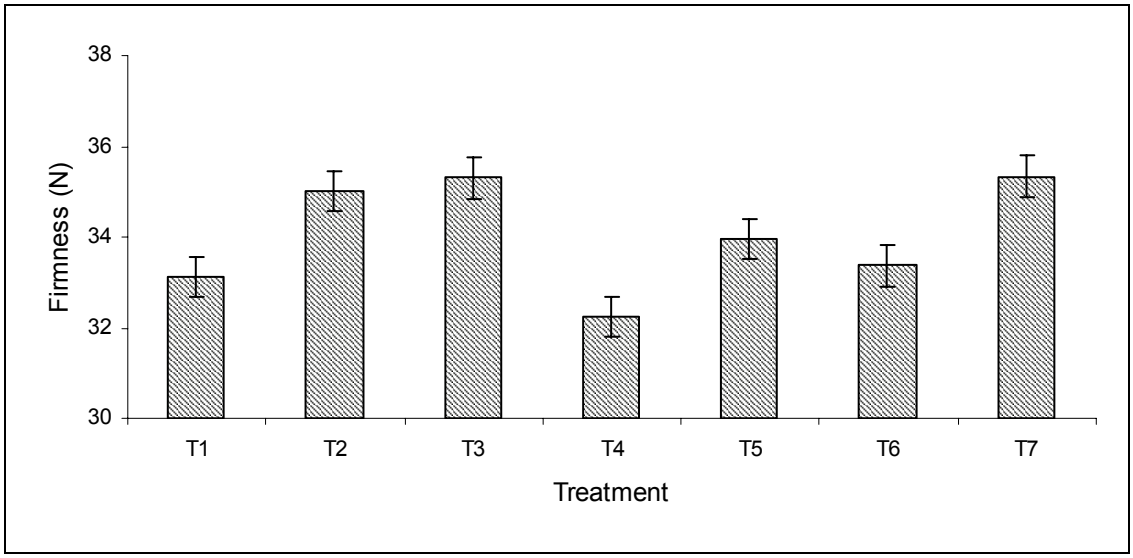

Figure 5.4: The effect of 2,4-D (T1), GA_3 (T2), and 6-BA (T3) on softening. The hormone concentration in the infiltration solution were zero (D-mannitol infiltrated (T4), water infiltrated (T5), evacuated non infiltrated (T6) and control fruits (T7)).

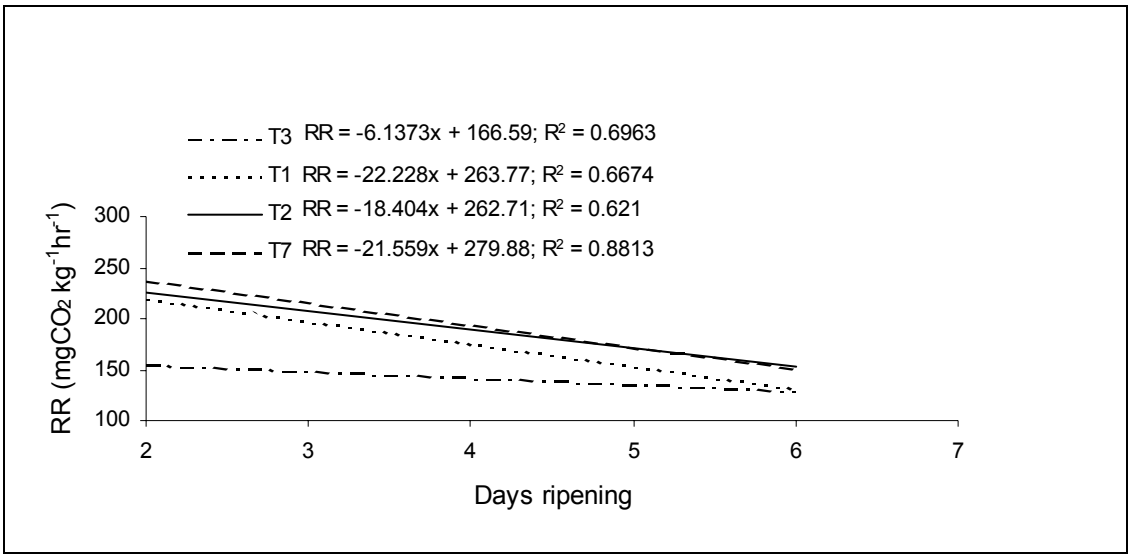

Figure 5.5: The effect of 2,4-D (T1), GA_3 (T2) and 6-BA (T3) on the respiratory pattern of 'Hass' avocado fruit. The hormone concentration in the infiltration solution were zero for the control fruits (T7).

Cytokinin application stimulated ethylene production in postclimacteric avocado fruit. These results differ from previous reports (Lieberman et al., 1977) where treatments of postclimateric avocado tissue with another cytokinin (Isopentenyl adenosine IPA), suppressed ethylene production. However, these authors suggested that if ethylene production in tissue slices of ripening fruits is an index of aging, then IPA would appear to retard aging in ripening fruit, just as other cytokinins appear to retard aging in senescent leaf tissue.

Figure 5.6. shows the effect of hormone application on ethylene evolution. The stimulation of ethylene synthesis in avocado fruit by hormone application was observed. However, enhanced ethylene evolution could not overcome the inhibition of degreening, softening, and the respiratory activity by phytohormones. On the contrary, there was an inverse relationship between the observed levels of ethylene and the acceleration of ripening.

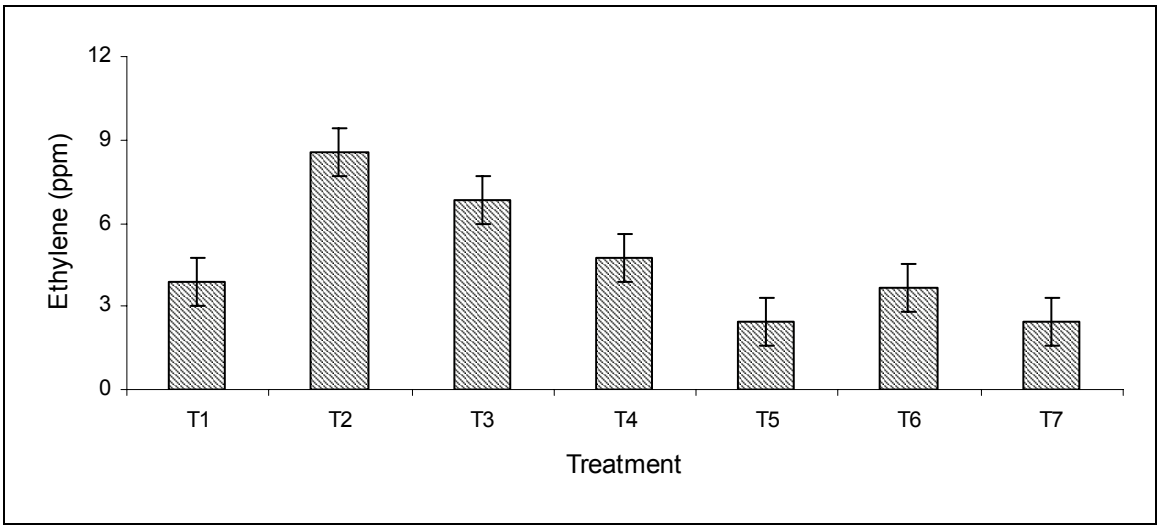

Figure 5.6: The effect of 2,4-D (T1), GA_3 (T2) and 6-BA (T3) on the average of ethylene production on the fourth and fifth day of ripening of treated 'Hass' avocado fruit. The hormone concentration in the infiltration solution were zero (D-mannitol infiltrated (T4), water infiltrated (T5), evacuated non infiltrated (T6) and control fruits (T7)).

The stimulative effect of auxin, gibberellin and specially cytokinin on ethylene production in avocado fruit did not represent an acceleration of aging but rather a specific responses of the fruit to supra optimal levels of hormone. There is evidence that auxins tend to counteract the aging process in some fruit tissues on the climacteric rise and in postclimacteric stages of senescence, despite its stimulation of ethylene production. Vendrell (1969) demonstrated this behaviour in banana and Frenkel and Dyck (1973) in Bartlett pears. In avocado, delay of ripening with auxin at much lower concentration levels than the level we used not accompanied by increased ethylene production (Tingwa and Young, 1975). The results observed here confirm that auxins can delay both ripening and respiration even though ethylene production increased.

Frenkel et al., (1969) suggested that the osmotic concentration of the infiltration solution played an important role in terms of fruit ripening behaviour. Fruits infiltrated with water and 0.3 M D-mannitol ripen more slowly in terms of softness, than non-infiltrated

fruits. Colour change and flesh softening were inhibited by water infiltration indicating that a favourable osmotic balance is required for ripening to occur. The high respiration rate of water-infiltrated fruit, as suggested by Frenkel et al., (1969), may be indicative of uncoupling of oxidative phosphorylation. Adato and Gazit (1974) found a negative linear correlation between the rate of daily water loss from harvested avocado fruit and the rate of ripening. Infusion of water delayed ripening, and they concluded that moisture stress is a determining factor in the rate of ripening.

Flesh softening, colour change and ethylene synthesis were not adversely affected by D-mannitol infiltration. Vacuum treatment of avocado fruits without infiltration of water or aqueous solution caused a significant increase in respiration rate. Tingwa and Young (1975) attributed the high respiration rate of evacuated fruit to either cell injury, or a sudden increase in availability of oxygen to an accumulated oxidizable substrate. This experiment suggests that various ripening parameters such as degreening, softening, respiration, and ethylene production are influenced by auxins, gibberellins, and cytokinins.

The data reported herein indicates that it is feasible to retard or even reverse senescence in bulk fruit tissue by supplying growth regulators. The penetration and distribution of the growth regulator within the fruit by vacuum infiltration of avocado fruit, provided even distribution and retarded ripening.

The rise of respiration rate in whole fruits is a phenomenon associated with aging and senescence; it is well known that cold and controlled atmosphere storage reduce the respiration rate of the stored commodity, retarding the ripening process. Consequently, the ability of hormones to alter respiration rate could be viewed as their ability to alter the aging process. It is conceivable that the resistance to ripening by avocado fruit at early stages of maturity or attached to the tree, reflects the action of inhibitory levels of auxins, gibberellins and cytokinins in the fruit tissue.

5.5. Conclusions.

The effect of cytokinin (6-BA) on avocado ripening is greater than that of the other hormones studied. Cytokinins are likely to be the substances (inhibitors) that prevent ripening of unpicked or freshly harvested avocado fruits. The significance of an optimum balance between cytokinins, other hormones and minerals for the effect to be shown cannot be discounted. More work is needed on the study of the endogenous content and evolution during ripening.

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CHAPTER VI

GENERAL SUMMARY, CONCLUSIONS AND RECOMENDATIONS.

"Hass" avocado, as all climacteric tropical fruits, has a limited shelf life due to its high metabolic activity. Decreasing the rate of ripening sufficiently to allow for shipping and marketing at a low cost requires understanding of the fruit's physiology and behaviour to different available treatments, storage methods and conditions.

In this study different combinations of modified and regular atmosphere storage were evaluated at different storage temperatures and with or without chemical or hormonal treatments.

The chemical treatment was limited to sulphur dioxide known for its antimicrobial, anti-oxidizing and anti-browning properties on fruits. The sulphur dioxide treatment is a non invasive one; it requires being in contact with the atmosphere surrounding the product.

The hormonal treatment included several plant hormones that required to be forced into the product. Since "Hass" avocado fruits have thick and pebbled skin, an experimental procedure for vacuum infiltration was needed. A procedure was devised and tested.

The avocado fruit was subjected to vacuum while submerged in the liquid to be infiltrated. The submersion in the liquid continues as the vacuum pressure is progressively released to return to pressure surrounding the fruit to atmospheric conditions. As the pressure within the system is increased, the air or gas returning to the interior of the produce will draw liquid with it and force the liquid through the stem (vascular system).

During vacuum infiltration, a permanent replacement of intracellular gases by liquid media results, which creates a longer lasting and easier to maintain internal modified atmosphere.

From this study we can conclude that the best treatment for storage of 1kg of 'Hass' avocado fruit was the silicon membrane system (28 cm^2) and low temperature 7°C with sodium metabisulphate treatment.

Sulphur dioxide seems to delay colour changes and softening of fruit; further research should be done to determine the optimum amount required per kg of product to enhance avocado storage.

The extent of cytokinins (6-BA) on avocado ripening exceeds that of the other hormones studied. Cytokinins are likely to be the substances (inhibitors) that prevent ripening of unpicked or freshly harvested avocado fruits.

The significance of optimum balance between cytokinins, other hormones and minerals for the effect to be shown cannot be discounted. More work is needed on the study of the endogenous content and evolution during ripening.

Vacuum infiltration on postharvest treatment studies to improve the quality and extend the shelf life of fresh produce should continue and be combined with other storing methods. The infiltrated liquid will carry with it any active soluble materials dissolved within. Examples might include probiotics, potassium, vitamins or even plain water (to increase moisture content). It is to be understood that any food grade material can be infiltrated by this system for any suitable chemical purpose, such as a fungicide, insecticide, colouring agent, preservative or taste modifier.

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