THE APPLICATION OF A MATHEMATICAL MODEL TO THE DESIGN OF MODIFIED ATMOSPHERE PACKAGING FOR MINIMALLY PROCESSED VEGETABLES

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ABSTRACT

Mathematical models were applied to the design of modified atmosphere packages for minimally processed vegetables, specifically, celery stalks, peeled garlic and onion slices. A review of the modified atmosphere packaging models developed by other researchers showed that many of the models were very similar, but the major problem encountered were the lack of suitable respiration rate data and optimum modified atmosphere composition data.

A modified atmosphere package for the celery stalks was developed using a simple model that relied on literature information for the recommended modified atmosphere gas composition. Respiration rate data were not required for the determination of the best package design. An optimum package design was found, and by the application of this simple model, other bag sizes could be designed that would be able to maintain the desired modified atmosphere.

For the design of the peeled garlic package, respiration rate data was determined in a closed system using an experimental design technique called Response Surface Methodology. The respiration data generated by this experiment was used in a mathematical model to design the required packages. Subsequent storage trials were unsuccessful, raising doubts about the accuracy of the respiration rate data. A modified atmosphere package was designed using the same method used to design the package for the celery stalks.

Response Surface Methodology was unsuccessfully applied to the determination of respiration rates for onions (sliced to various thicknesses) using a closed system. The use of a dynamic model of a package of sliced onions was also unsuccessful in determining the respiration rates. Further work needs to be undertaken to evaluate experimental design techniques for determining the respiration rates of minimally processed vegetables. This data can then be used in mathematical models to determine the optimum modified atmosphere package design.

The importance of processing hygeine is stressed, with high microbial numbers growing within four days on untreated minimally processed produce, but little growth after washing in antimicrobial solutions before packaging.

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Thomas R. Robertson May, 1992

TABLE OF CONTENTS

TABLE OF CONTENTS CONTINUED: PAGE

TABLE OF CONTENTS CONTINUED:

PAGE

CHAPTER 5 CONTINUED:

LIST OF TABLES

LIST OF TABLES CONTINUED:

LIST OF TABLES CONTINUED:

LIST OF FIGURES

LIST OF FIGURES CONTINUED:

LIST OF FIGURES CONTINUED:

CHAPTER 1

INTRODUCTION

1.1 Introduction

The national and international markets for prepared and chopped salads and vegetables are rapidly expanding. Chopped or shredded plant tissues deteriorate more rapidly and thus have a shorter shelf-life than produce marketed in a "uncut" condition (Geeson & Brocklehurst, 1989). The smaller pieces of tissue are more susceptible to moisture loss and wilting, and the cut surfaces dry out and sometimes discolour. The juices from the damaged cells at the cut surfaces provide an excellent medium for the growth of microorganisms. These can rapidly grow to high numbers leading to spoilage, off-odours and the increased risk of growth of food-poisoning bacteria. Of particular concern are those bacteria able to grow at the low storage temperatures at which these semi-processed vegetables are stored, like *Listeria monocytogenes,* and *Clostridium botulinum* type E (Brody, 1989).

Studies at various laboratories around the world have demonstrated the advantages of suitably designed modified atmosphere (MA) packaging systems in slowing deteriorative changes, while extending the shelf-life of various whole fruits and vegetables (Geeson & Brocklehurst, 1989). There is thus considerable benefit to be gained by the food industry and the consumer if similar packaging methods and designs could be developed to improve the safety, quality, and shelf-life of minimally processed produce.

To achieve the required shelf-life for the minimally processed vegetable produce at the anticipated storage temperatures, the following major variables are under the producer's control (Geeson & Brocklehurst, 1989):

- Use of pre-treatments to reduce the initial microbial load by washing and the use of special sanitizers. Pre-treatments can also include anti-browning preparations.

- The choice of packaging materials and pack construction. The rate of modification of the pack atmosphere is influenced by a number of factors including weight and respiration rate of the produce, and the surface area, thickness, and gas permeability of the packaging material. The selection of the film type, method of pack construction,and the ratio of fill weight and film area affect the changes that occur in the gas composition of the pack.

- The use of evacuation and gas flushing techniques. The composition of the pack atmosphere will be modified, but the rate of modification may be too slow at chill temperatures to retard deterioration sufficiently. The use of gas flushing equipment to provide the required atmosphere at the point of packing may be required.

The interactions of all the various factors in the design of processing and packaging procedures for minimally processed vegetables can be seen in Figure 1.1

1.2 Modified Atmosphere Package Design

Packaging of produce in polymeric films is a common technique designed to prevent moisture loss, to protect against mechanical damage, and to provide better appearance. The proper selection of packaging films and optimizing package design can favourably alter the gas composition around fruits and vegetables, resulting in an extended shelf life and improved quality. A produce package is a dynamic system in which two main processes, respiration and permeation, are occurring simultaneously, resulting in modified atmosphere compositions.

These modified atmospheres (MA) result from the removal or addition of gases resulting in an atmosphere surrounding the commodity that is different from that of air (78.08% N_2, 20.95% O_2, 0.03% CO_2). Usually this involves reduction of oxygen and/or elevation of carbon dioxide concentrations. These changes in the atmosphere surrounding the food product may retard some of the normal deteriorative reactions (eg spoilage due to oxidation, senescence, staling, enzyme activity). Developments have occurred in the application of modified atmospheres, where the gas composition is controlled only at the point of packaging. In such packs some change of gas composition will occur during shelf-life, depending on such factors as the evolution or absorption of gases by the food, the permeability of the packaging materials, seal integrity and storage temperature. This is Modified Atmosphere Packaging (MAP) and this study will investigate packaging models that predict the equilibrium atmosphere resulting within a package given the commodity mass and respiration rate and the film size and permeability to O_2 and CO_2.

Chapter 2 will cover the critical elements of a packaging model and then compare this with models that have already been developed. Figure 1.2 presents a model of the food commodity and its environment, showing some of the reactions that occur in a modified atmosphere package. Subsequent research uses parts of the packaging model developed by Mannapperuma and Singh (1990) to design MA packages for celery stalks, peeled garlic and sliced onion. A major part of this research is the determination of the optimum modified atmosphere for these minimally processed vegetables, except in the case of the celery stalks when is was assumed that the celery stalks approximated whole celery and the recommended modified atmosphere from literature sources was used. The mathematical model was applied in a different way to assist in the design of the modified package for the celery stalks.

The research to develop the modified atmosphere packages for the celery stalks and peeled garlic was partially funded by commercial companies and the design of the final packs should be considered confidential.

The objectives of this study are:

Figure 1.1 Factors to Consider in the Design of Processing and Packaging Procedures for Minimally Processed Produce (Geeson & Brocklehurst, 1989)

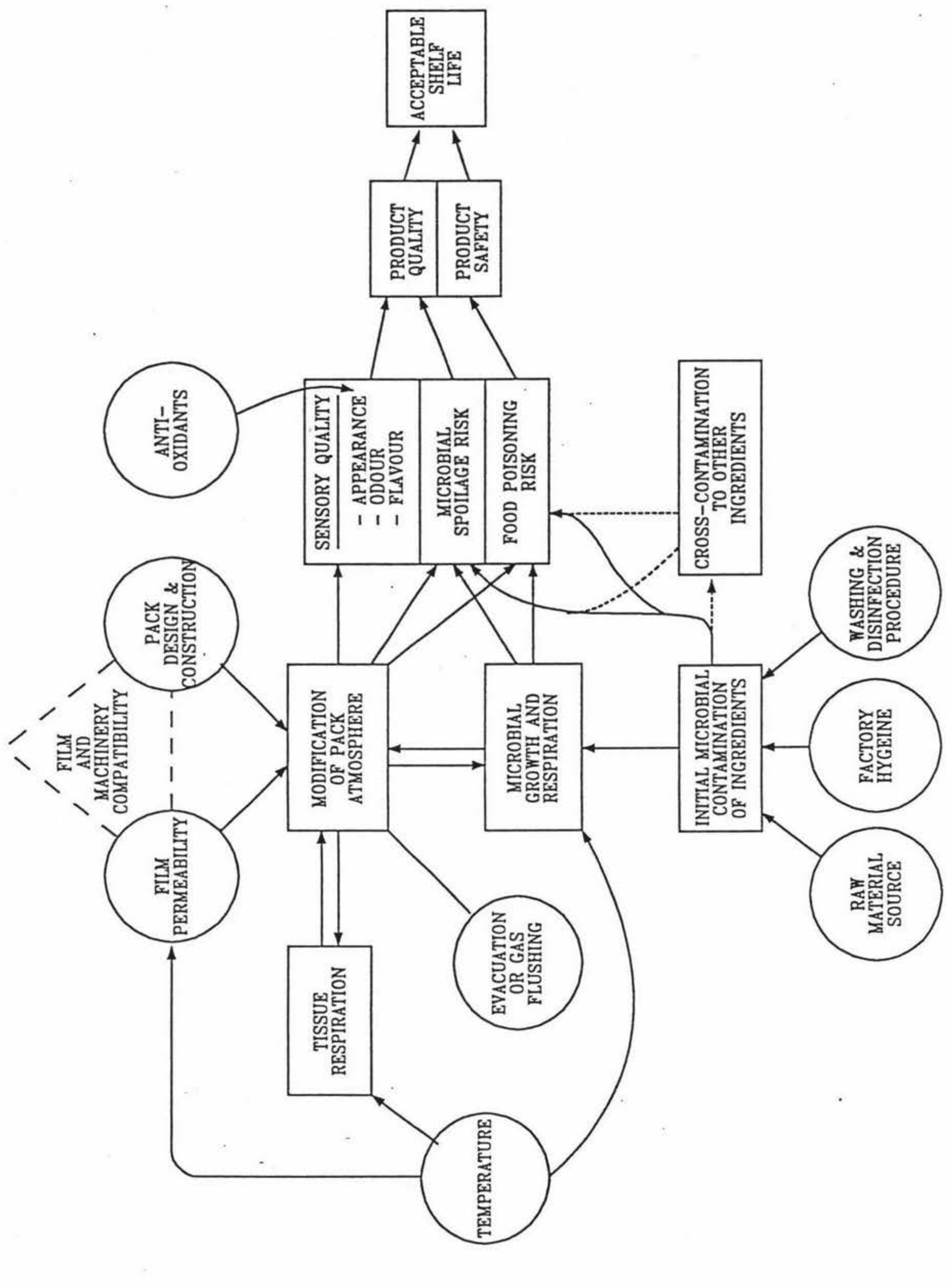
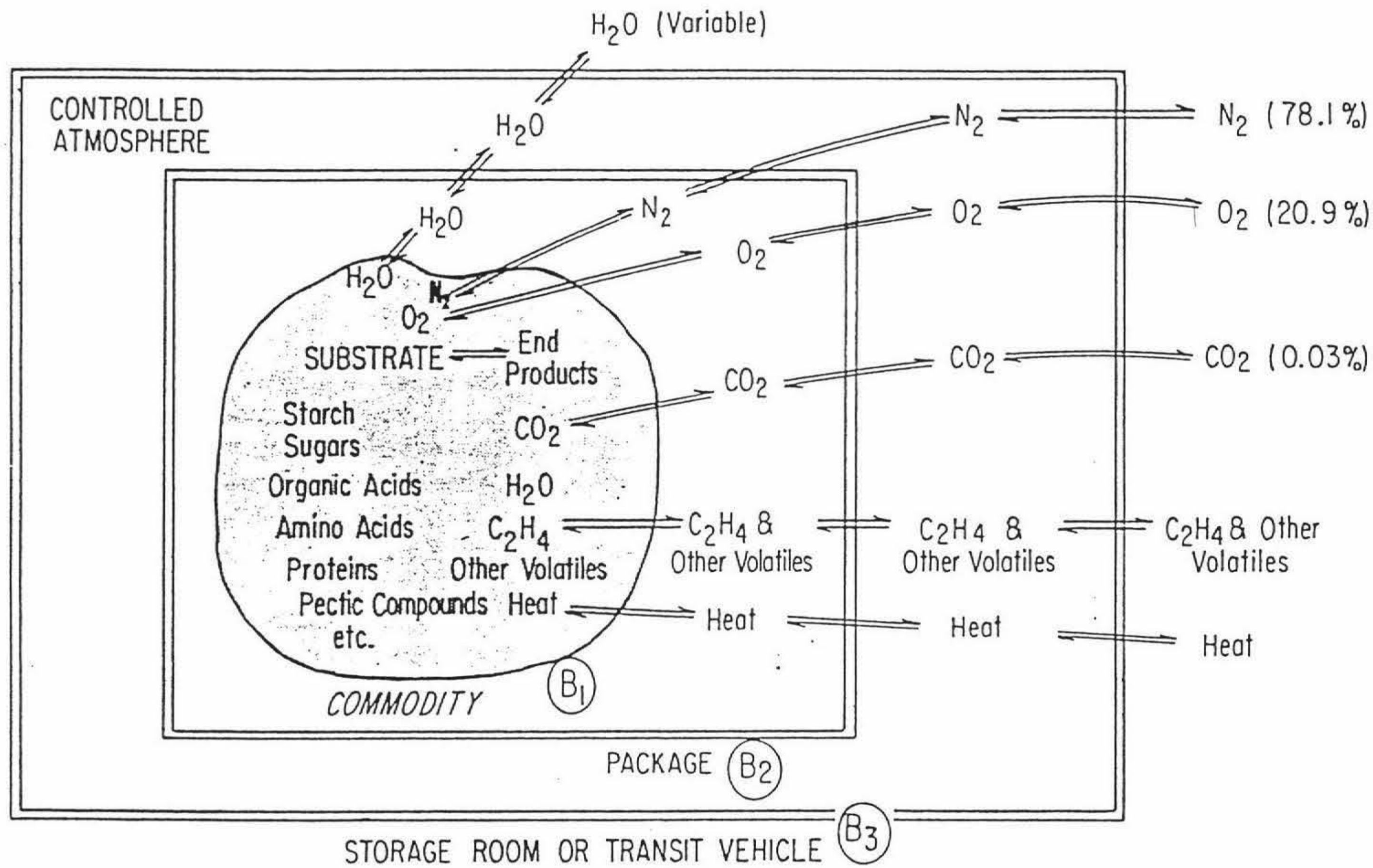


Figure 1.2 A Model of the Commodity and its Environment Showing the Types of Barriers which can be Used to Establish a Modified Atmosphere.(Kader, 1988)



Types of barriers which can be used to establish a modified atmosphere.

B_1—Natural epidermis, skin, peel, or rind. Wax coating, film wrap.

B_2—Package—Wood, paperboard, plastic (may include additional liner in package).

B_3—Storage room wall or vehicle wall, may be sealed against gas exchange.

Additional barriers may include—consumer packages inside the master package and pallet covers over several packages.

1) To evaluate the parts of the mathematical model from Mannapperuma and Singh (1990) to develop modified atmosphere packaging for sliced onion, peeled garlic and celery stalks.

2) To evaluate an experiment design technique called Response Surface Methodology to determine the required modified atmosphere for the peeled garlic and the sliced onion.

CHAPTER 2

MODIFIED ATMOSPHERE PACKAGING MODELS

2.1 'Optimum' Model Requirements

Kader, Zagory and Kerbel, (1988) presented information on the requirements of a Modified Atmosphere Model (MAP) model. The information is set out under four headings:

a) determination of gas diffusion rate in the commodity
b) determination of respiration rate of the commodity
c) film characteristics and permeability
d) external factors

2.1.1 Determination of the Gas Diffusion Rate of the Commodity.

The following information should be available to ensure that an accurate determination of the gas diffusion rate in the commodity is obtained:

a) respiration rate
b) maturity stage
c) physiological age
d) commodity mass and volume
e) pathways and barriers for diffusion
f) properties of the gas molecules
g) concentration of gas in the atmosphere around the commodity
h) magnitude of gas concentration gradient across barriers
i) temperature.

2.1.2 Determination of the Commodity Respiration Rate

To ensure the correct respiration rate is determined the following information should be considered and used:

a) kind of commodity
b) maturity stage
c) physical condition of the commodity
d) concentration of carbon dioxide, oxygen, and ethylene in the package
e) commodity quantity in the pack
f) temperature
g) light (for some commodities).

2.1.3 Film Characteristics and Permeability

The correct selection of packaging material requires the following information:

a) film structure
b) film permeability to specific gases
c) film thickness
d) surface area of film
f) concentration gradient across the film
g) temperature
h) pressure differentials across the film
i) relative humidity (for some films).

2.1.4 External Factors

The important external factors are temperature and maybe light. This could be extended to include time/temperature profiles of the distribution and retail chain, and conditions of storage and of domestic use of the product. This latter aspect appears not to be covered by other authors, and yet is very important if the packaged products are to be successful in the marketplace.

2.1.5 Critical Elements of a Packaging Model.

Kader et al. (1988) summarise the requirements of a packaging model as being able to predict the equilibrium atmosphere resulting within a package given the commodity mass respiration rate and the film size and permeability to oxygen and carbon dioxide. The atmospheric changes should also be predicted over time and estimate how long until equilibrium is established. To do this the model must incorporate commodity size, gas-diffusion resistance and the headspace volume of the package. Application of a packaging model requires the following information (summary of sections 2.1.1 - 2.1.4 above) (Kader et al., 1988):

a) The dependence of respiration on O_2 concentration.
b) The dependence of respiration on CO_2 concentration. Carbon dioxide can have a variable effect on the respiration of all commodities and the model should have some empirical data as a guide.
c) Commodity limiting factors, i.e., minimum concentration of O_2 before anaerobic conditions cause fermentation and the maximum concentration of CO_2 before injury occurs.
d) Permeability of the polymer films of interest and the effect of temperature and humidity on permeability.
e) The diffusion resistance of the commodity to O_2, CO_2, and C_2H_2.
f) The Respiration Quotient (RQ) for a given product and any changes that may occur as the atmosphere changes.
g) The optimal atmosphere for storage of a given product.

It is possible that such a model could predict the type of packaging film required for a particular commodity.

The following models have been developed and used over the last 30 years and each model is compared to the above requirements to assess its ability to predict the required parameters. All the equations in the text have been modified to use the same symbols as those used by Mannapperuma and Singh (1990).

2.2 Tolle (1962)

Tolle (1962) designed a model using regression equations and extrapolations of apple respiration data from 82 references to develop a model of film permeability requirements necessary to generate the desired MA in bushel boxes of apples lined with film.

The following factors were used in this model:

a) respiration rate
b) packaging type
c) internal volume of the package and its surface area
d) partial pressure differences of O_2 and CO_2
e) the change in respiration rate to specific O_2 & CO_2 concentrations, expressed as % of normal respiration rate
h) permeability of the films to water vapour.

Tolle (ibid.) noted the importance of the following but did not include them directly into the model:

a) effect of oxygen and carbon dioxide concentration changes on the respiration rate, except for the empirical treatment in (e) above
b) that biological factors should be considered when selecting the type of film to use

The respiration rates used were from empirical data and Tolle used only polyethylene and parchment as the packaging materials.

The model proposed by Tolle (ibid.) for calculating the CO_2 and O_2 permeability requirements was:

a) For Carbon Dioxide:

$$R_2.s.a.v.(x_2 - c_2) = P_2 \qquad (2.1)$$

b) For Oxygen:

$$R_1.s.a.v.(c_1 - x_1) = P_1 \qquad (2.2)$$

where

R	=	respiration rate constants
s	=	box-liner surface constant
a	=	package atmosphere constants for CO_2 & O_2

v = package volume
c = gas concentrations in ambient atmosphere
x = gas concentrations in the package atmosphere
P = film permeability
suffixes 1 and 2 denote oxygen and carbon dioxide respectively

The constants 'R', 's' & 'a' are derived from the reference data studied by Tolle (ibid.) and are detailed in his paper.

The units Tolle (ibid.) used for polymer film permeability were:
mL CO_2/m^2/24 hrs/760 mmΔ/storage temp (°F)
and mL O_2/m^2/24 hrs/760 mmΔ/storage temp (°F)

where mmΔ is the partial pressure difference.
The model was relatively simple but it did point the way to later developments in MA packaging models by showing that such models were possible and workable within the limits Tolle (ibid.) was working with.

The major problem encountered by Tolle (ibid.) was the poor quality of the packaging material he was using. Pinholes, uneven thicknesses and thus poor knowledge of the films' permeability to gases, made accurate prediction of MA conditions difficult. This model does not predict the changes in pack atmosphere, but can be used to calculate the permeability of the film required to maintain a desired atmosphere for apples. This calculated permeability takes no account of the actual permeability of available polymer films. Tolle (ibid.) did not evaluate the effectiveness of his model using fresh apples or other produce, so it is difficult to assess how accurate the model was.

Thus Tolle's model was based on the products of respiration rate, gas partial pressure differentials, film surface area and an empirically derived package-volume adjustment and package-atmosphere constant.

2.3 Jurin and Karel (1963)

Jurin and Karel (1963) used a gas chromatographic method to study the respiration rates of Macintosh apples as a function of oxygen concentration. The influence of carbon dioxide concentration on the relationship between oxygen pressure and respiration rates was also studied. Estimates were obtained on the critical concentrations of oxygen and carbon dioxide that result in the onset of anaerobic respiration. The relationships obtained in the respiration studies were used for prediction of optimum packaging conditions for the apples. The packaging parameters considered were volume, surface area, and permeability to oxygen and carbon dioxide. The predicted relations between time of storage and gas exchange under given packaging conditions were tested in short-term storage studies. The results obtained were considered to be useful in evaluating package requirements for fresh fruits and vegetables.

Assuming the steady state concentrations of O_2 and CO_2 are in the range in which the CO_2 content was determined by this research to have no effect on the rate of respiration, and in which the respiratory quotient (RQ) is equal to 1.0, the resulting conditions may be represented by the following equations:

$$V_c = f(x_1) \tag{2.3}$$

Where V_c = volume of oxygen consumed (mL/package x day)
x_1 = oxygen concentration in the package expressed as a fraction of the atmosphere concentration

$$V_d = P_1 \frac{A}{b}(c_1 - x_1) \tag{2.4}$$

Where
V_d = oxygen diffusing into package (mL/package x day)
P_1 = oxygen permeability of the packaging material (mL)(mil)/(m^2)(day)(atm)
A = area of the package (m^2)
b = thickness of the packaging material (mil)
c_1 = partial pressure of the oxygen in the atmosphere surrounding the package (atm)
x_1 = partial pressure of oxygen in the package (atm)

Jurin and Karel (ibid.) then assumed steady state conditions (V_c is equal to V_d) and that the inside and outside of the package are at a total pressure of 1 atmosphere. Then x_1 has the same value in both equations, and equations 2.3 and 2.4 can be rewritten as follows;

$$V_c = V_d = f(x_1) = P_1 \frac{A}{b}(c_1 - x_1) \tag{2.5}$$

Note: that in these steady state conditions V_c and V_d would equate to the weight of produce and the produce's oxygen respiration rate, i.e. WR_1. If the analytical expression for $f(x_1)$ is known, the equations may be solved simultaneously to a give steady state value of the oxygen concentrations in the package.

This model is an empirical method for determining the equilibrium O_2 concentration for a given film and product. The model assumes an RQ of one and that the CO_2 concentration within the hypothesized package is too low to affect fruit respiration. Jurin and Karel (ibid.) plotted product respiration as a function of O_2 concentration on the same set of coordinates as film permeation. The intersection of the two curves represents the equilibrium O_2 concentration.

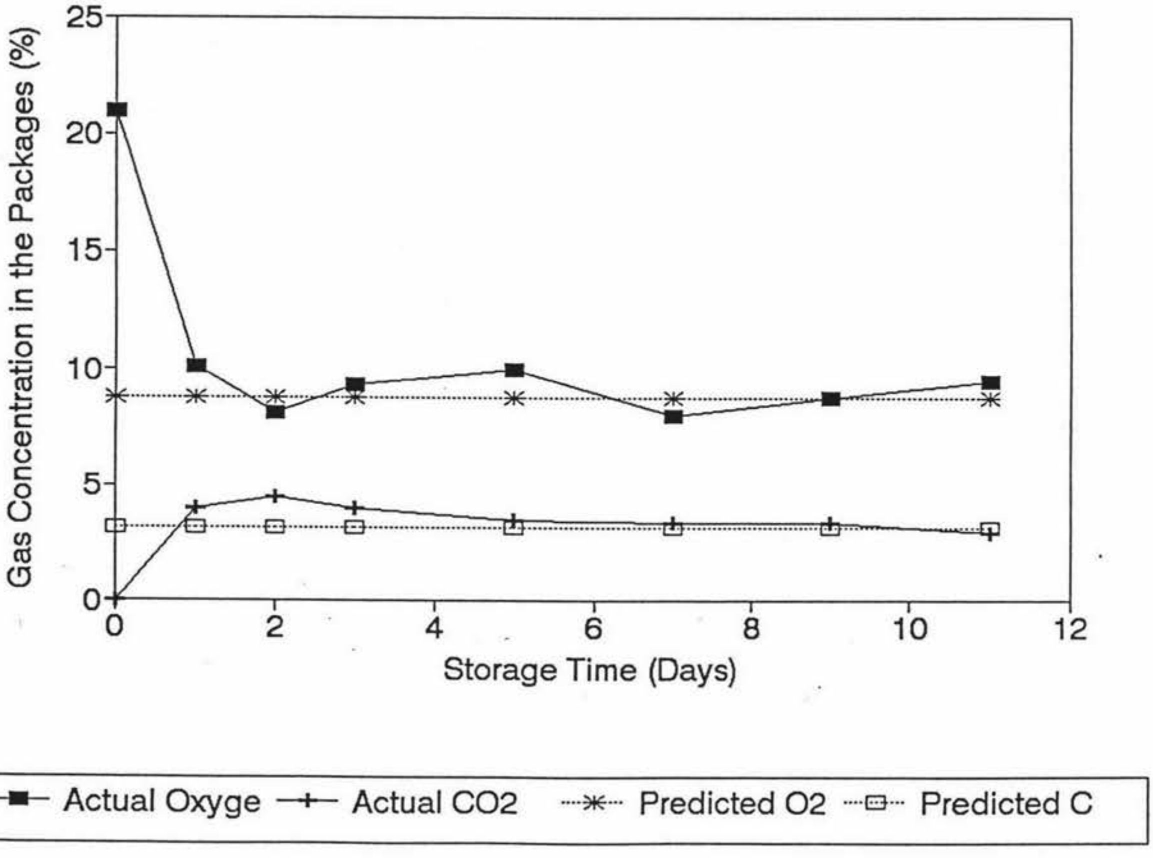
The factors considered in the model include:

a) gas partial pressures
b) package surface area
c) package permeability to the gases O_2 and CO_2
d) effect of CO_2 concentration on respiration rate
e) steady state conditions
f) RQ = one

For the model to work Jurin and Karel (ibid.) chose to work with a RQ of 1 even though their own research had demonstrated that at low concentrations of oxygen (below 4%), there was a rise in the RQ. The model is only able to predict the steady state gas concentrations in the pack (Figure 2.1) and not how the atmosphere changes with time. Thus while this model is useful in predicting the steady state gas concentrations in the final pack, it should provide sufficient information to allow pack design for commercial MAP operations. The model requires a large number of calculations to determine which packaging material is required to provide the required package atmosphere, as it would have to be used with all the different materials until the appropriate polymer film is identified.

This model for predicting the oxygen equilibrium is still quite simple in respect to the factors being considered.

Figure 2.1 Comparison of Predicted and Observed Concentrations of Oxygen and Carbon Dioxide in Polyethylene Bags Containing Apples, (from Jurin & Karel (1963)).



2.4 Veeraju and Karel (1966)

Veeraju and Karel (1966) presented an analytical model to design packages by calculating the film areas and the conditions for film selection. Two films were used (low density polyethylene and vegetable parchment, of different permeability characteristics in order to independently control the O_2 and CO_2 concentrations in the package. The model used the following factors:

a) permeability of the two films used
b) surface area of the film
c) thickness of the film
d) weight of product
e) rate of O_2 consumption
f) rate of CO_2 production
g) RQ of 1

This model was adequate to design a package to maintain a desired equilibrium atmosphere, but did not cover the processes leading up to the equilibrium. The authors noted that many factors affected the accuracy of the predictions. These include:

a) the inherent variations in the respiration rates of different lots of fruit
b) change of respiration rates with time
c) variations in the thicknesses of films used
d) variations in the permeability of films made from the same material
e) small changes in room temperature, which considerably affect respiration rates

The authors also noted the effect of fruit maturity and the effect of ripening on the respiration rates. Problems were still being encountered by Veeraju & Karel (ibid.), with variable polymer films, a problem that has largely disappeared with today's improved polymer technology.

The model does provide a very useful method that can be used to combine two polymer films to produce a package with the required overall permeability to CO_2 & O_2. This was achieved by manipulation of the following two equations (2.6 and 2.7), which can determine the volume of O_2 & CO_2 respectively passing through the film:

$$W(c_1-x_1) = \left[\frac{A_a P_{a_1}}{b_a}+\frac{A_b P_{b_1}}{b_b}\right]\frac{20.8-R_1}{100} \tag{2.6}$$

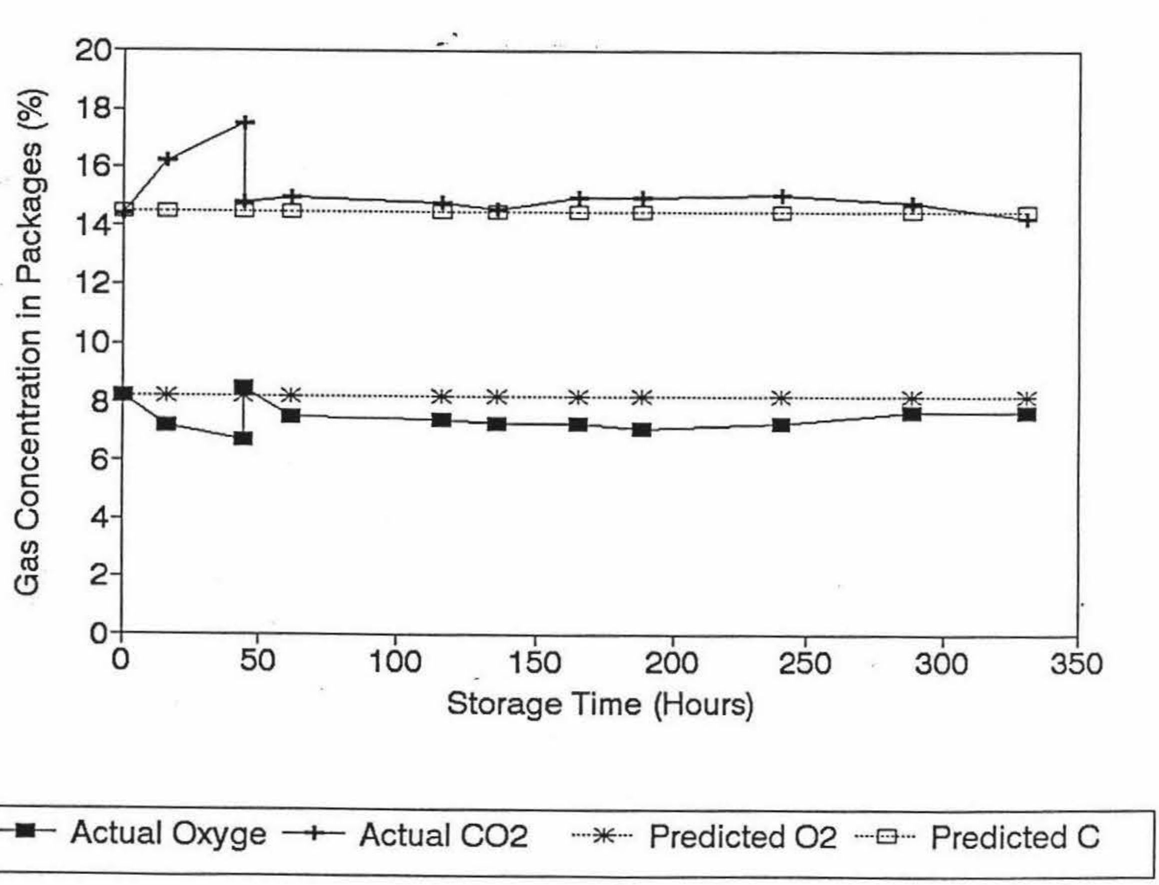
$$W(x_2-c_2) = \left[\frac{A_a P_{a_2}}{b_a}+\frac{A_b P_{b_2}}{b_b}\right]\frac{R_2}{100} \tag{2.7}$$

Where

W	=	weight of fruit
$(c_1 - x_1)$	=	rate of consumption of oxygen (mL/kg.day) at steady state conditions
$(x_2 - c_2)$	=	rate of consumption of carbon dioxide (mL/kg.day) at steady state conditions
A_a & A_b	=	areas of films A and B (m^2)
P_{a1} & P_{a2}	=	permeability of film A to oxygen and carbon dioxide (mL. mil/m^2 day atm)
P_{b1} & P_{b2}	=	permeability of film B to oxygen and carbon dioxide (mL. mil/m^2 day atm)
b_a & b_b	=	thickness of films A and B (mils.)

Veeraju and Karel (ibid.) also recommended the selection of the proper films based on the ratio of their CO_2 and O_2 permeabilities. The effectiveness of the model in predicting the steady state concentrations in the package can be seen in Figure 2.2. The actual steady state concentrations varied considerably from the predicted after 44 hours, at which point Veeraju and Karel (ibid.) recalculated the areas of the films by using different respiration data. The resulting package maintained similar gas concentrations compared to the predicted values, as illustrated in the later stages of the storage trial (Figure 2.2). The accuracy of the literature values for respiration rates was thus a problem in applying this model, although the respiration rates could be recalculated and the package redesigned during the storage trials.

Figure 2.2 Comparison of the Predicted and the Experimentally Observed Gas Concentrations (from Veeraju and Karel (1966))(Note: the package was redesigned after 44 Hours).



2.5 Massignan (1987)

Massignan (as quoted by Kader et al. (1989) - the original reference was unavailable) developed a steady state model to predict package oxygen and carbon dioxide concentrations for eggplant using an empirically derived linear regression equation to estimate respiration under any set of gas concentrations. The model accurately predicted equilibrium concentrations for eggplant packaged in three films.

All of the above models assume post-climacteric or non-climacteric fruit, equilibrium or steady state conditions and an RQ equal to one. The use of fruit undergoing the rapid changes in the respiration rate that occur during the climactic period of the fruit made modelling these very complex. Similarly the changes that occur in the atmosphere leading up to the steady state conditions are also more complex but the authors may have been concentrating on practical models for commercial use, where the changes in the atmosphere are less important than the steady state atmosphere. The last assumption of an RQ of one was selected for the ease of model development, but this does limit the use of the models especially when the package atmosphere has low oxygen levels and high carbon dioxide levels. The following models attempt to address some of these issues.

2.6 Marcellin (1974)

Marcellin (1974) used estimates of RQ and gas partial pressures to calculate the sizes, thicknesses and the permeability coefficient of polyethylene bags suitable for wrapping various fruit and vegetables (including artichokes, carrots, asparagus, and turnips and green peppers). The model was extended to describe a polyethylene package that contained a silicone rubber window to increase gas permeability. The model assumed equilibrium conditions and post-climacteric or non-climacteric fruit or vegetables. The factors considered in the model included:

a) respiration quotient
b) film permeability
c) optimal atmosphere
d) package area.

This model provides information on the changes to the atmosphere that occur inside the package while taking into account the changes that can occur in the respiration quotient. The main thrust of the model is to optimise the area of the silicone window to ensure a good control of the required gas concentrations inside the bag. In trials conducted using artichokes and asparagus at 0°C, carrots and turnips at 1°C to 2°C, and green peppers at 12°C to 13°C, the model successfully calculated the required window size of silicone rubber, to maintain the required gas atmosphere for the asparagus, artichokes and the green peppers. The design for the carrots and turnips was less successful as a result (according to Marcellin (ibid.)) of water condensation inside the bag. A comparison of the gas composition inside the package of artichokes (Figure 2.3), where the package was successfully designed, with the gas composition inside the package of carrots (Figure 2.4), where the model designed package was less successful, would indicate that some or all of the following factors used in the package design may be incorrect:

a) respiration rates
b) window size selected (it allowed too much oxygen to enter the package atmosphere)
c) weight of carrots in the package.

This model was developed to assist in the design of commercial bulk Controlled Atmosphere packages up to pallet size.

Figure 2.3 The Changes in Gas Composition in a Package of Artichokes, Designed by Marcellin's Model, During Storage Trials (from Marcellin (1974)).

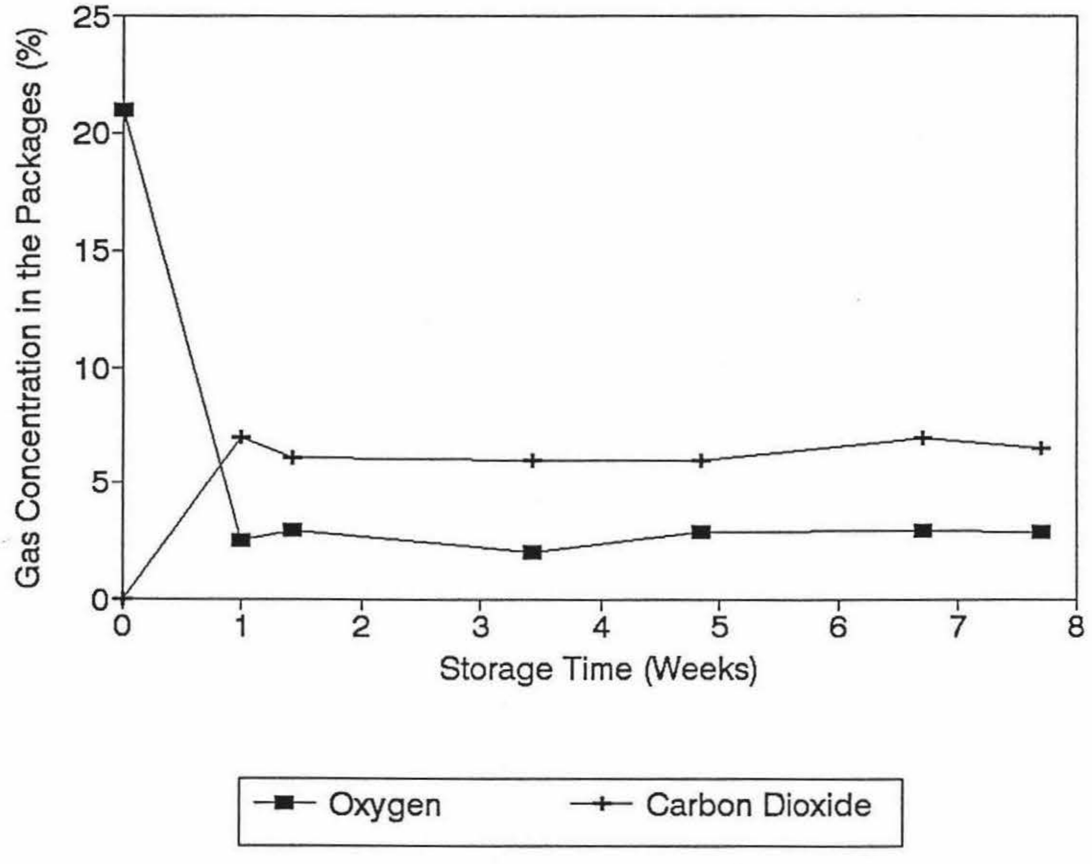
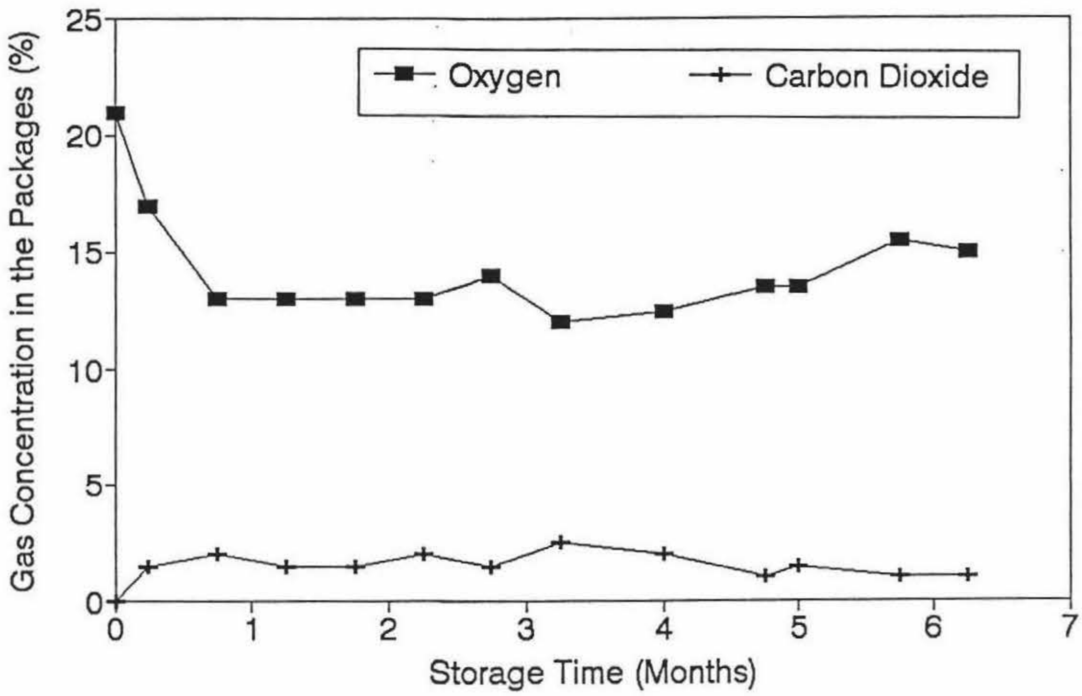


Figure 2.4 The Changes in Gas Composition in a Package of Carrot , Designed by Marcellin's Model, During Storage Trials (from Marcellin (1974)).



2.7 Kok and Raghavan (1984)

Kok and Raghavan (1984) developed a mathematical model of a Marcellin type modified atmosphere storage system for apples. This system depends on the combined actions of the respiration of the fruit and the differential diffusion of several gases through a thin membrane to attain a desirable gas composition in the storage system, where most of the storage unit is of a non-permeable material i.e. a coolroom with a polymer membrane diffusion unit.

The model includes the following factors:

a) surface area of the polymer membrane
b) thickness of the polymer membrane
c) CO_2 and O_2 respiration rates of the apples i.e. CO_2 generation rates and O_2 consumption rates
d) storage volume (of the coolroom, not just the free volume)
e) polymer permeability to CO_2, O_2 and N_2

The model assumes:

a) RQ of one for model simplicity
b) all the apples are the same, and
c) the temperature and gas composition throughout the storage area are uniform.

The authors determined the rate of CO_2 generation as a function of temperature and of the partial pressures of O_2 and CO_2 in the gas phase. Respiration data from different sources were then used, but these data were very scattered making modelling difficult.

This model deals with a type of modified atmosphere storage as applied to a bulk storage situation, but could have application to container loads of apples. This paper, while published in 1984, has no references later than 1973, and has no reference to Marcellin's model described above. The difficulty in using this model lies in obtaining and using the relevant respiration data, especially as it is the respiration rate function that plays the crucial role in modelling the storage of the apples. Kok & Raghavan (1984) provided information on the changes in variables, as predicted by the model, that occur in a Marcellin storage system, but did not compare the results with an actual storage trial. Thus it is difficult to assess the effectiveness of the model.

2.8 Henig and Gilbert (1975)

Henig and Gilbert (1975) developed a computer-aided solution to the mathematical equations representing the changes in the respiratory gas concentrations within tomato packages. The authors state that "the technique enables rapid prediction of the O_2 and CO_2 concentrations within produce packages, taking into account all the packaging variables. The technique also provides a good tool for package design for any commodity without extensive experimentation or field trials"

The model consists of two simultaneous first order ordinary differential equations together with boundary and initial conditions. They solved the mathematical models through the use of a simple finite difference procedure, and then investigated the influence of several packaging parameters on the transient and equilibrium state gas compositions in sample packages. There are no packaging parameters explicitly included in the solutions and these have to be optimised through trial and error. The model predicted what the atmosphere composition in the package would change to but no consideration was given to the optimum O_2/CO_2 concentrations for the tomatoes to maximize shelf life.

This model assumed a variable RQ and transient gas concentrations, and uses first-order differential equations with numerical solutions to optimize package parameters. They did however assume that environmental CO_2 had little effect on the rate of O_2 consumption, and environmental O_2 had little effect on CO_2 production, and thus did not include these factors in their model. This assumption was made even though Henig and Gilbert experimentally demonstrated a significant reduction in O_2 consumption with increasing CO_2 concentrations. It was contended that as the change was small it did not affect the model. While this may have been the case with tomatoes, other fruit and vegetables may not be affected the same and this reduction in O_2 should be considered. The model included the following factors:

a) package headspace
b) package surface area
c) time to steady state conditions

d) film permeability to O_2 and CO_2
e) effect of O_2 on respiration

The two first-order differential equations representing the system are:

$$\frac{dx_1}{dt} = P_1 A(0.21 - \frac{x_1}{V}) - f(\frac{x_1}{V}, \frac{x_2}{V}) \quad (2.8)$$

$$0\frac{dx_2}{dt} = g(\frac{x_1}{V}, \frac{x_2}{V}) - P_2 \frac{x_2}{V} A \quad (2.9)$$

Where

x_1	=	volume of O_2 in the package (mL)
x_2	=	volume of CO_2 in the package (mL)
V	=	total free volume in the package (mL)
P_1	=	permeability of the film to O_2 (mL/hr.in^2)
A	=	area of the packaging (in^2)
P_2	=	permeability of the film to CO_2 (mL/hr.in^2)
t	=	time (min)
f	=	a function representing O_2 consumption rate
g	=	a function representing CO_2 evolution rate.

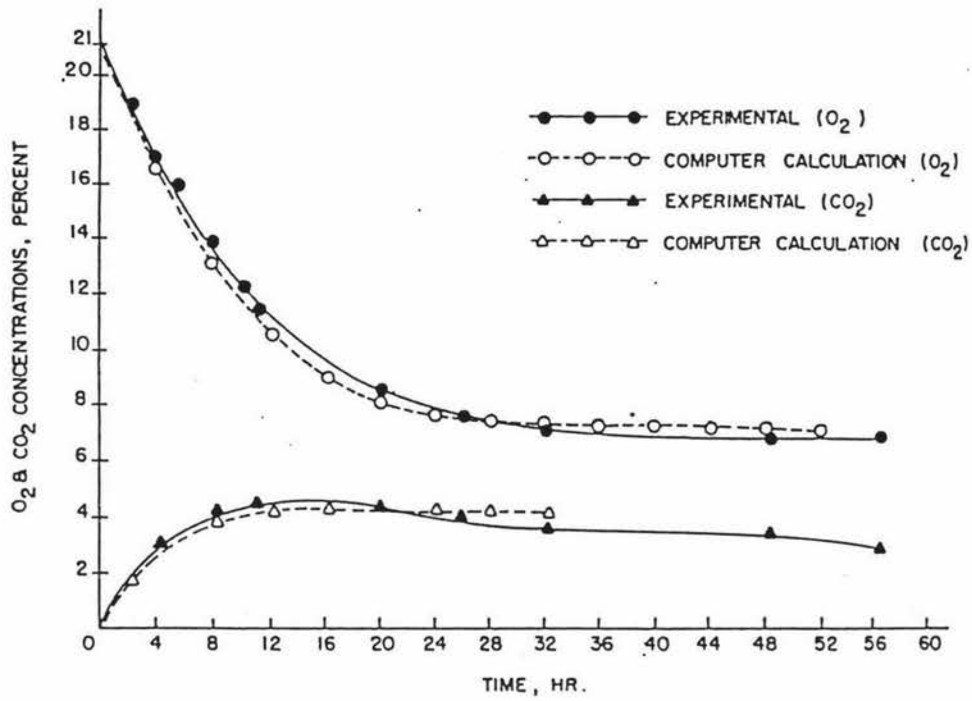
assuming the functions f and g can be replaced by R_1W/V and R_2W/V, gives equations 2.10 and 2.11.

$$V\frac{dx_1}{dt} = P_1 A(0.21 - \frac{x_1}{V}) - R_1 W \quad (2.10)$$

$$V\frac{dx_2}{dt} = R_2 W - P_2 A \frac{x_2}{V} \quad (2.11)$$

and where $(0.21 - x_1/V)$ equates to $(c_1 - x_1)$ and (x_2/V) to $(x_2 - c_2)$
This model was only evaluated using tomatoes in a plasticized polyvinyl chloride film and the theoretical solution compared very well with the experimental results (Figure 2.5). The model was not extended to designing packages using other films or produce.

Figure 2.5 A Comparison Between Henig & Gilbert's model calculations and the Experimental Results of the Atmosphere Change in a Plasticized Polyvinyl Chloride Film Package Containing Tomatoes (from Henig & Gilbert (1975)).



2.9 Hayakawa, Henig & Gilbert (1975)

This model is a development of Henig and Gilbert's model after careful examination of respiration data (Hayakawa et al. 1975). The new mathematical model was developed for simulating the gas exchange of a fresh produce package. Analytical solutions are obtained that are then used in simple algebraic formulae that derive the optimum packaging parameters. The major difference between the two models is that Hayakawa et al.'s model reflects the effects of both O_2 and CO_2 concentrations on O_2 consumption and CO_2 evolution. Hayakawa et al. (ibid.) used the following factors in their model:

a) Gas diffusion

- i) respiration rate
- ii) concentration of gases in surrounding atmosphere
- iii) transit values of CO_2 and O_2
- iv) maturation stage
- v) commodity mass & volume

b) Respiration Rate

i) concentration of CO_2 and O_2 in the package (estimated)
ii) kind of commodity
iii) maturity stage
iv) weight of produce (estimated)

c) Film

i) thickness
ii) permeability
iii) surface area
iv) inside volume of package
v) polymer type.

The analytical formulae can be used to estimate:

a) transient and steady state gas concentrations in fresh produce packages
b) equilibrium time values for the gas exchange of the sample packages
c) CO_2 and O_2 equilibrium concentrations

There was fair agreement between the estimations and experimental results for the transient and steady state gas concentrations in fresh produce packages, and CO_2 and O_2 equilibrium concentrations. There was also agreement between the estimates and experimental results for the time to equilibrium for O_2. However, there were big differences between CO_2 estimates and experimental time to equilibrium values. The authors think this was due to assumptions imposed for experimentally determining the respiration rate constants of fresh produce. The limiting factor in this model is the method used to calculate the produce respiration rates using respiration rate curves which are mathematically complex.

For package design using this model, Hayakawa et al. (ibid.) simplified their model, which required complicated calculations, by assuming in post-climacteric fruit:

a) O_2 respiration was not affected by the CO_2 concentration in the package; and
b) the rate of CO_2 evolution was not significantly affected by O_2 concentrations in the surrounding package.

These assumptions allowed for easier calculation of the packaging parameters. By modifying the equation given in Hayakawa et al. (ibid.) for calculating the permeability required such that ($y_{eql}o_i + q_i$) and ($z_{eql}e_i + f_i$) are functions describing the produce O_2 and CO_2 respiration, then equations 2.12 and 2.13 can become equations 2.14 and 2.15.

$$\frac{P_1}{b} = \frac{(y_{eqi}o_i+q_i)\frac{W}{A}}{(c_1-x_1)} \qquad (2.12)$$

$$\frac{P_2}{b} = \frac{(z_{eqi}e_i+f_i)\frac{W}{A}}{(x_2-c_2)} \qquad (2.13)$$

By replacing the respiration functions that Hayakawa et al. (ibid.) used [$(y_{eqi}o_i + q_i)$ and $(z_{eqi}e_i + f_i)$] with R_1 and R_2, then equations 2.12 and 2.13 become:

$$WR_1 = P_1 A \frac{(c_1-x_1)}{b} \qquad (2.14)$$

$$WR_2 = P_2 A \frac{(x_2-c_2)}{b} \qquad (2.15)$$

Where

A	=	area of film (in^2)
R	=	respiration rate (mL/hr.kg)
P	=	permeability coefficient(mL/hr.in^2 (atm/mil))
W	=	weight of produce in the package (kg)
c	=	gas concentrations in ambient atmosphere (atm)
x	=	gas concentrations in package atmosphere (atm)
b	=	thickness of the film (in)

suffixes 1 & 2 denote oxygen and carbon dioxide gases repectively

Hayakawa et al. (ibid.) have introduced complex mathematics to calculate the appropriate produce respiration rates for use in polymer package design.

2.10 Deily and Rizvi (1981)

The objective of the model developed by Deily & Rizvi (1981) was to optimize the parameters for packaging of produce in permeable materials during the constant respiration rate period. The model tried to integrate commodity variables such as respiration rate, weight and optimum gaseous composition requirements with packaging parameters like permeability, surface area and free volume. The model can predict transient and equilibrium time values for O_2 and CO_2 concentrations within a produce package, or it can be used to design the package when the gaseous composition is known.

Deily & Rizvi (ibid.) do, however, assume that changing O_2 concentrations between 21% and 5% and CO_2 concentration between 0% and 25% are not reflected in changes in respiration rate. This empirical assumption was based on data for fresh peaches, although it is not clear what conditions were used to generate these data. This assumption would have to tested for other produce before the model could be applied to that produce. The model uses the following factors:

a) oxygen transmission rate of a polymer film
b) carbon dioxide transmission rate of a polymer film
c) rate of consumption of oxygen
d) rate of evolution of CO_2
e) surface area of produce package
f) time after packaging
g) free volume inside the package
h) produce weight
i) volumetric concentration of O_2 gas inside the fresh produce package.

The model also looked quantitatively at the effect of storage on quality parameters of the fruit, i.e. firmness, soluble solids and cohesiveness of the flesh. According to the authors the actual and predicted values for different films and for a given set of package parameters were similar and within experimental error. The optimum package for the peaches could not be created as there were no commercially available polymer films with the model generated CO_2 and O_2 permeability rates.

A later refinement of this model (Rizvi et al. (1987)) was based on a mass balance of permeating gases with variable produce respiration rates. The resulting model predicted the effect of package headspace, polymer permeability and product characteristics on internal gas concentrations.

2.11 Lakin (1987)

Lakin (1987) described a computer program called Computer Aided Hermetic Package Design (CAHPD) based on the dynamics of package barrier insufficiency for either oxygen or moisture sensitive foods. This model has not been designed to develop packaging systems for respiring commodities, but for non-respiring foods. This model would require considerable modification before it could be used to predict MAP packaging requirements or changes in modified atmospheres in the packages.

2.12 Cameron, Boylan-Pett and Lee (1989)

Cameron et al. (1989) developed a model based on the determination of respiration data for tomatoes at three different stages of maturity. They determined the O_2 consumption as a function of O_2 concentration by mathematical characterization of O_2 depletion by the tomatoes in a closed system. The model is a continuous mathematical function which can be used to develop novel predictive models for optimization of O_2 in the package, and for predicting the permeability characteristic of a polymeric film required to maintain a given O_2 concentration for a range of tomato weights.

The model assumes:

a) constant temperature
b) no influence of CO_2 concentration on the O_2 respiration rate, and
c) includes no CO_2 production rate data.

The model includes the following factors:

a) diffusive flux of O_2 per unit time through the film
b) permeability coefficient of the film
c) surface area of the film
d) thickness of the film
e) O_2 partial pressure outside the package
f) O_2 partial pressure inside the package.
g) flux of O_2 into the fruit as a function of the respiration rate which is a function of the O_2 concentration within the package
h) weight of fruit in the package.

The model concentrated on package design and the steady state O_2 concentration and did not consider changes in the atmospheric concentrations inside the package. The RQ variations were not considered in this model, with CO_2 concentrations kept below 0.1%. Equation (2.16) was developed to predict the required permeability of the film for different weights of fruit; it has practical application in pack design for MAP with respect to O_2.

$$\frac{P_1 A}{b} = \frac{q(1-e^{-r(x_1)})^s . W}{(c_1 - x_1)} \tag{2.16}$$

Where

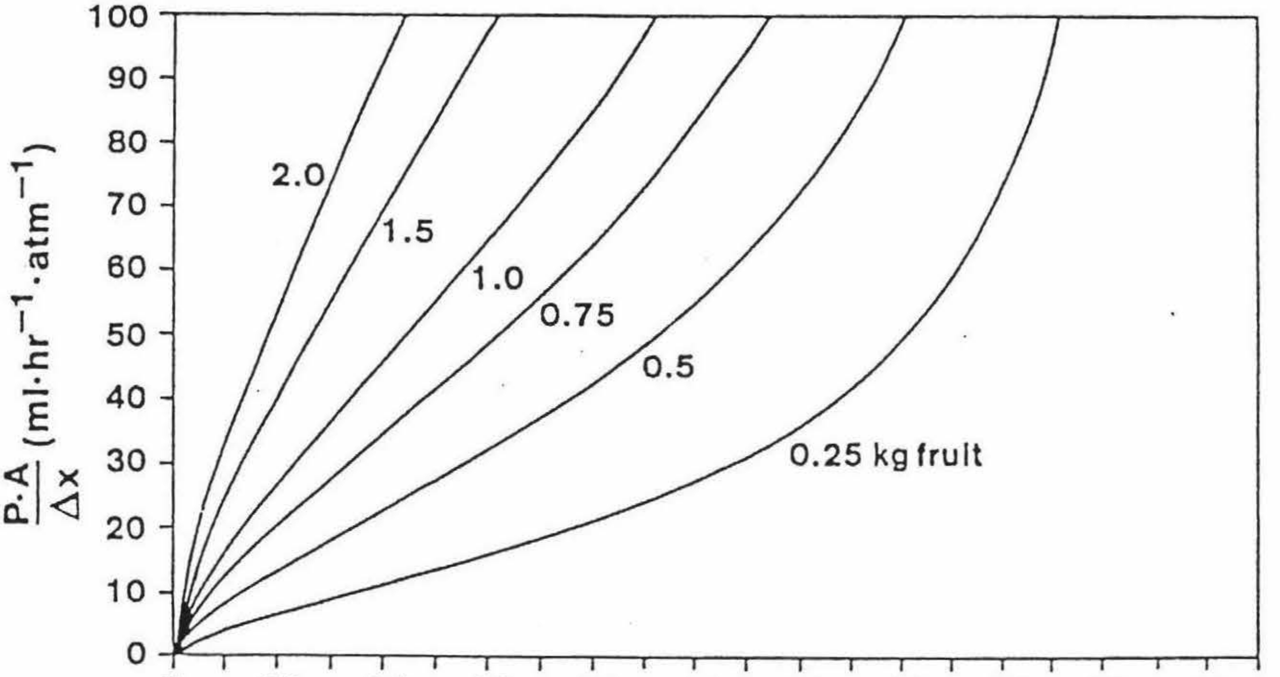
P_1	=	O_2 permeability coefficient of film
A	=	surface area of film
b	=	film thickness
q, s & r	=	equation constants relating to respiration of O_2
x_1	=	partial pressure of O_2 within the package
W	=	weight of fruit in package
c_1	=	partial pressure of O_2 outside package

Thus $q(1 - e^{-r x1})^s$ is a function describing O_2 respiration and can be replaced with R_1 to produce equation 2.17.

$$\frac{P_1 A}{b} = \frac{R_1 W}{(c_1 - X_1)} \tag{2.17}$$

This equation can be used to produce graphs (Figure 2.6) that can be used to predict the required polymer permeability for a given weight of tomatoes in a package of known size. This part of the model would be very useful for the commercial tomato packer trying to determine the type of polymer to use for different weights of tomatoes for a modified atmosphere package.

Figure 2.6 Predicted permeability characteristics of a polymeric film required to maintain a given concentration of O_2 for a range of weights of red tomatoes at 25°C in a sealed package environment. (from Cameron <u>et al.</u> (1989))



2.13 Cameron (1990)

Cameron (1990) further developed the above model to include carbon dioxide, with the steady state O_2 and CO_2 concentrations being used to calculate the rate of flux through the package, which for a functioning modified atmosphere pack should be equal to the rate of respiration of the packaged fruit. The model was then applied to tomatoes and cherries, the model predicting the O_2 uptake versus O_2 concentration reasonably accurately. It was less accurate at predicting the rate of O_2 uptake at lower O_2 concentrations and the rate of CO_2 production.

The method used to test this model was a type of controlled pack-and-pray approach in which different fruit masses were placed in a single design package. These single design packages were preferred since it is well known that published permeability values are not always correct (Cameron, 1990).

The model does not take into account the effect of CO_2 concentration on the O_2 uptake. The model does allow the determination of the optimum concentration of O_2 in the package environment for extended shelf-life. Optimization of the concentration of CO_2 was considered limited by the lack of appropriate films.

2.14 Yang and Chinnan (1988)

Yang and Chinnan (1988) developed an integrated model to predict gas composition and colour development of tomatoes stored in polymeric film packages throughout the storage period. The main aim of the model development was to allow selection of the appropriate polymeric films for modified atmosphere storage.

The model concentrates on describing the effects of gas composition and storage period on respiration rate. The model was tested using only one cultivar of tomato and thus may not be applicable to other types of fruits and vegetables. Yang and Chinnan further suggest that as respiration is an enzyme-mediated process, models based on enzyme kinetics theory may be more appropriate. However, considering the effect of environmental gaseous composition makes the whole modelling process very complex.

This model and the one developed by Cameron (1990) have concentrated on the problem of obtaining the appropriate respiration rate data for developing modified atmosphere packages.

2.15 Mannapperuma et al. (1988)

A summary of this model is set out in Zagory and Kader (1988) and in more detail in Mannapperuma et al. (1989) and Mannapperuma and Singh (1990) with application in Zagory et al. (1989). It is intended for general use for any fresh fruit or vegetable. The model uses respiration rates at several oxygen concentrations and to allow simulation of changes in the atmosphere in a package of specified dimensions and permeability, assumes respiration kinetics follow a linear combination of zero and first order behaviour. The model predicts transient and equilibrium gas concentrations both within the package and the produce (these values may differ due to different gas diffusion rates of commodities).

The model can be used to select polymer film types if the permeabilities are known, or calculate the desired permeability coefficient of the film for a particular commodity. The model assumes that the RQ is equal to one and uses temperature explicitly as a variable, but other variables are measured at specified temperatures. The effect of elevated CO_2 concentrations on respiration rate has not been included. Any effect is, however, implicitly included in the data for respiration at different O_2 concentrations by using respiration rate data generated in the presence of elevated CO_2 combined with reduced O_2. This model uses published respiration rate data and recommended modified atmospheres. The following factors are used in the model:

a) package headspace
b) package surface area
c) change-over time
d) film permeability to O_2 and CO_2
e) effect of O_2 and CO_2 on respiration
f) produce diffusion resistance
g) optimal atmosphere
h) RQ of 1 or greater than one

A simpler mathematical model, one that assumes post-climatic fruits and a respiration rate of one, was selected by Mannapperuma and Singh (1990) to illustrate the physical significance of many design parameters. This model is set out in equations 2.18 and 2.19.

$$WR_1 = P_1 A \frac{(c_1 - x_1)}{b} \tag{2.18}$$

$$WR_2 = P_2 A \frac{(x_2 - c_2)}{b} \tag{2.19}$$

Where

A = area of film (m^2)
R = respiration rate (moles/kg.s)
P = permeability coefficient (m^2/s)
W = weight of produce in the package (kg)
c = gas concentrations in ambient atmosphere (moles/m^3)
x = gas concentrations in package atmosphere (moles/m^3)
b = thickness of the film (m)
suffixes 1 & 2 denote oxygen and carbon dioxide gases

Note that equations 2.18 and 2.19 are the same as those used in other models. This model equates steady state gas flow rates to equilibrium respiration rates of the produce.

Mannapperuma and Singh (ibid.) developed this model to study the effect the eleven variables present in package design by rearrangement of equations 2.18 and 2.19 together with the introduction of some new parameters.

According to Mannapperuma and Singh (1990) in the design of packages, the type of produce determines R_1 and R_2, as well as determining the recommended modified atmospheric composition x_1 and x_2. This selects the type of film and hence P_1 and P_2, leaving the package designer with the choice of only three variables: thickness of the film, area of the package, and the weight of the produce in the package. The method of selecting these three variables can be shown by rewriting equations 2.18 and 2.19 and introducing a new parameter ϕ.

$$x_1 = c_1 - \frac{R_1}{P_1}\phi \qquad (2.20)$$

$$x_2 = c_2 + \frac{R_2}{P_2}\phi \qquad (2.21)$$

where $$\phi = \frac{Wb}{A} \qquad (2.22)$$

This model allows for easy application to the design of appropriate modified atmosphere packages provided the correct respiration rate data is available. Mannapperuma and Singh (ibid.) also discuss two different methods of measuring the respiration rate, and a method can be developed from their dynamic model:

a) Using an open system with a steady stream of the gas mixture of the required composition passed over the produce in a jar until equilibrium is reached;

b) Using a closed system with the produce kept in a closed container and the gas composition inside the container analzyed over a period of time. The oxygen and carbon dioxide respiration rates at any instant are calculated by mass balance on gas components using equations 2.23 and 2.24 respectively.

$$R_1 = \frac{V}{W}\frac{dx_1}{dt} \qquad (2.23)$$

$$R_2 = \frac{V}{W}\frac{dx_2}{dt} \qquad (2.24)$$

Where

R	=	respiration rate (moles/kg.s)
W	=	weight of produce in the package (kg)
dx	=	change in gas concentrations in container atmosphere (moles/m^3)
dt	=	time interval (seconds)
V	=	free volume in the container (m^3)

suffixes 1 & 2 denote oxygen and carbon dioxide gases respectively

c) Using a dynamic model to analyze the changes in the atmosphere inside a polymer package containing produce (see Chapter 6 for the detailed equations).

2.16 Model Comparison

A comparison of the different models is set out in Table 2.1. The factors listed are those deemed important by Kader et al. (1988) and each model is compared with this 'ideal'. A more general summary of the models is detailed in Table 2.2, showing the major area covered by each model.

Table 2.1: Summary of Factors Included in Mathematical Models of Modified Atmosphere Packaging of Fruit and Vegetables.

	PROPOSED MODELS									
FACTORS	1	2	3	4	5	6	7	8	9	10
RESPIRATION RATE DETERMINATION:										
Effect of O_2 on respiration	+	-	+	+	-	+	+	-	+	
Effect of CO_2 on respiration	+	-	+	+	-	+	+	-	+	
Commodity type	n			n	n	-	+		+	
Maturity stage	-		-	-	n	-	+		+	
Physical condition	n		-	-	-	-	+			
Quantity commodity in package	+		+	-	+	+	+	+	+	
Temperature	+	+	-	n	+	-	-	-	+	
GAS DIFFUSION DETERMINATION:										
Product diffusion resistance	-	-	-	-	-	-	-	-	+	
Respiration rate	+		+	+	n	+	+	+	+	
Maturity stage	-		-	-	n	-	+	-	+	
Physiological age	-		-	-	n	-	+	-	+	
Commodity mass and volume	+		+	-			+		+	
Properties of gas molecules	-		-	-	-	-	-	-		
Concentration of gas in atm around commodity	-		-	+	-	-	+	-	+	
Magnitude of gas conc. gradient across barriers	-		-	+	-	-	-	-		
Temperature	+	+	-	-	-	-	-	-	+	
FILM CHARACTERISTICS & PERMEABILITY:										
Film permeability	+	+	+	+	+	+	+	+	+	+
Temperature	+	+	-	-	-	-	-	-	+	
Package area	+	+	+	+	+	+	+	+	+	+
Package head space	+	+	-	n	-	+	+	+		
Film structure	+		-	-	-	-	-	-		
Conc. gradient across film	-		-	-	-	-	-	-		
Relative humidity	-		-	-	-	-	-	-		
Pressure differentials across film	+		+	-	+	-	-	-		
Film thickness	+		+	+	+	-	+	-		
OTHER FACTORS:										
Optimal atmosphere	-	-	-	-	+	-	-	-		
Change-over time	-	+	-	-	-	+	+	+		
Respiration quotient	-	-	-	-	+	-	-	-		

+ Partially used in model - Not used in model
n Noted, but not used in model
1) Tolle (1971) 2) Lakin (1987)
3) Jurin and Karel (1963) 4) Veeraju and Karel (1966)
5) Marcellin (1974) 6) Henig and Gilbert (1975)
7) Hayakawa et al. (1975) 8) Deily and Rizvi (1981)
9) Mannapperuma and Singh (1990)
10) Massignan (1987) - (Reference 1) [not enough information available]

Note: "Light" and "injurious gas levels" were not included in any of the above models.

Table 2.2 The Main Area Covered by the Models for Modified Atmosphere Packaging.

Model	Major Areas Covered by the Model			
	Packaging Design	Steady State	Changing Atmosphere	Respiration
Tolle, 1962	Commercial	Yes		
Jurin & Karel, 1963 limited	Yes			Yes
Veeraju & Karel, 1966	Yes - 2 films	Yes		
Massignan, 1987 limited			Yes	
Marcellin, 1974	Bulk packs		Yes	
Kok & Raghavan, 1985	Bulk stores	Yes		
Henig & Gilbert, 1975	Yes			Yes
Hayakawa et al., 1975	Yes			Complex
Deily & Rizvi, 1981 yes	Yes	Yes	(Yes)*	
Cameron et al., 1975	O_2 only			O_2 optimal
Cameron, 1990	Yes	Yes		
Yang & Chinnan, 1988	Film selection			Yes
Mannapperuma et al., 1988	Yes	Yes	Yes	
Mannapperuma & Singh, 1990	Yes	Yes	Yes	Calculation

* in a later model developed by Rizvi et al. (1987).

2.17 Research Needs

Most of the research in MAP has concentrated on finding the right package for the produce/product under study. What is required is a more comprehensive research approach where studies are based on a rational understanding of the principles and processes and where packaging attempts are developed from a conceptual model of MA. This will require an understanding of the interactions of:

a) temperature
b) O_2 and CO_2 concentrations
c) film permeability, and
d) commodity diffusion resistance, and their effect on quality parameters.

More information is required on:

a) film permeability at different temperatures and humidity;
b) respiration rates for fresh produce over a wide range of MA/CA conditions;
c) general relationships for the additive effects of decreasing O_2, increasing CO_2, and C_2H_4
d) effect of packaging on the equilibrium atmosphere directly rather than allowing the development of this equilibrium, something that the models studied have not taken into account;
e) changes in RQ as the atmosphere composition changes;
f) resistance of fruit and vegetables to diffusion of O_2, CO_2 and C_2H_4.

Optimum modified atmosphere also reduces ethylene production resulting in delayed senescence, usually indicated by retention of chlorophyll, textural quality and the sensory quality of non-fruit vegetables. The requirement to reduce ethylene production is particularly important for climacteric fruits, where exposure advances the onset of an irreversible rise in respiration rate and rapid ripening. Exposure of non-climacteric produce to ethylene can also increase the respiration rates, thus ethylene control is important in designing modified atmosphere packaging of some produce.

An area that could also be researched is the combination of the optimum conditions for minimally processed produce/commodity versus that for the contaminating microorganisms.

2.19 Concluding Remarks

The following comments from Chinnan (1991) summarize the current state of MAP models. "The fundamental equations describing the gas exchange within the horticultural commodities and between the micro-atmosphere inside the package and the environment outside the package are not very complex. However, the challenging aspect of the modelling process is:

a) obtaining solutions to equations while using realistic and meaningful assumptions, and
b) validation of the models with experimentally obtained data.

Researchers have been successful with developing models and validating them but the models are of limited scope. One of the limiting factors in developing models for modified atmosphere storage and package systems is the availability of respiration data in a form able to be used in the predictive methods."

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Celery

Celery (*Apium graveolens* var. *dulce*) was picked in the morning in Pukukohe, cooled to remove the field heat, and air freighted to Palmerston North for processing and packaging. On arrival the celery was broken down into separate stalks which were then cut to size by trimming off the leaves at the first junction and trimming the base.

The pre-preparation of the celery before packaging is critical to the success of the shelf-life trials, and the procedure used after the trimming was as described by Armstrong (1990):

i) The celery stalks were washed in cold water (less than 7°C, using ice in cold water) with mild agitation for sufficient time to remove most of the retained dirt or grime that may inactivate the chlorine. This required 3 to 5 minutes of washing.

ii) The celery was then washed in a second cold water bath (maintained at less than 7°C) containing about 200ppm active chlorine, buffered to a pH of 5 to 7 with potassium monobasic phosphate (about 1% concentration), for at least one minute. (0.622g calcium hypochlorite/litre of water was used to obtain 200ppm of active chlorine; 10g of potassium monobasic phosphate buffer per kg gave a final pH of 5.9).

iii) The treated celery was drained immediately and the adhering moisture removed using a basket centrifuge.

The celery was then ready for the packaging experiments and at this stage it was 24 to 30 hours after harvesting.

3.1.2 Garlic

The garlic (*Allium sativum*) was harvested, segmented into cloves and peeled by a farming group in Blenheim, cooled and then air-freighted to Palmerston North. On arrival, the garlic was put through the same wash sequence as the celery to remove any dirt and to reduce the microbial contamination on the peeled garlic cloves. These cloves where then used for the respiration and packaging trials, at which stage the garlic was less than 24 hours ex-peeling.

3.1.2 Onion

Onion (*Allium cepa* var. *cepa*) of a variety known as Pukekohe Long Keeper, a golden-brown skinned bulb with a very good shelf life, was used for all the trials.

These onions were purchased from a local supermarket for most of the experimental research. Peeled onions supplied by a commercial grower with an automatic peeling machine were used for the larger shelf life trials.

For the respiration experiments the onions were hand-peeled and then washed using the same cold washing sequence as described above. These onions were hand sliced using a ruler and a sharp knife to obtain the required slice thickness. For the bulk storage trials the onions were not washed and were sliced using a meat slicer to the required thickness.

3.2 Computer Programmes

3.2.1 Quattro Pro

Quattro Pro (Borland International, Scotts Valley, California, USA) is a computer spreadsheet programme developed by Borland with a similar lay out to Lotus 123 and VP Planner. Quattro Pro was used to calculated the respiration rates using Equations 2.23, 2.24, 6.5 and 6.6 developed by Mannapperuma and Singh (1990). The graph function of this programme was used to produce the graphs.

3.2.2 Response Surface Methodology Experimental Design Package

The Response Surface Methodology (RSM) Experimental Design Package (McKesson Technical Center, Dublin, California, USA) is an experimental design package that can test 2 - 5 variables simultaneously, calculate their linear and interaction effects on multiple responses, and then use the models to prepare three dimensional, response surface-type contour maps to describe the effects.

RSM has two types of experimental designs:

a) The "Exploratory" type (using five levels of each variable) highlights the most important variables for each measured response, and whether the chosen variables and their ranges will solve the problem.

b) The "Optimisation" type (using three levels) pinpoints optimum conditions more precisely. The model developed by RSM is based on a Taylor Second Order Equation.

The key steps in using the RSM programme are:

a) Identify the experimental goals, the responses used to describe the goals, and the 2 - 5 key variables.

b) Develop an experimental plan based on the variables and their ranges, and the type of experiment (Exploratory or Optimization).

c) Print out an experimental plan, in random order, and a log for recording the experimental results for each response.

d) Run the experiment.

e) Analyze the results to:
- calculate the Taylor Second Order Equations for each response.
- provide statistical calculations and confidence statements.
- produce X/Y graphs, using the Taylor Second Order Equations, to illustrate relationships over the entire experimental region.

3.2.3 Surfer

Surfer (Golden Software, Inc, Golden, Colorado, USA) is a graphics package able to produce 2-dimensional topographical and 3-dimensional graphs from experimental data. This programme was used to graph the garlic respiration rate data at the actual O_2 and CO_2 concentrations and produce the diagrams to higher standard than those produced by RSM.

3.3 Gas Composition Measurement

The gas composition of the atmosphere was measured using a method similar to that described by Jurin and Karel (1963). A Gas Chromatograph, SRI Model 8610-07, with 4-filament thermal conductivity detector and an Alltech CTR1 column ("column in column", molecular sieve/porous polymer) was used with the following operating parameters:

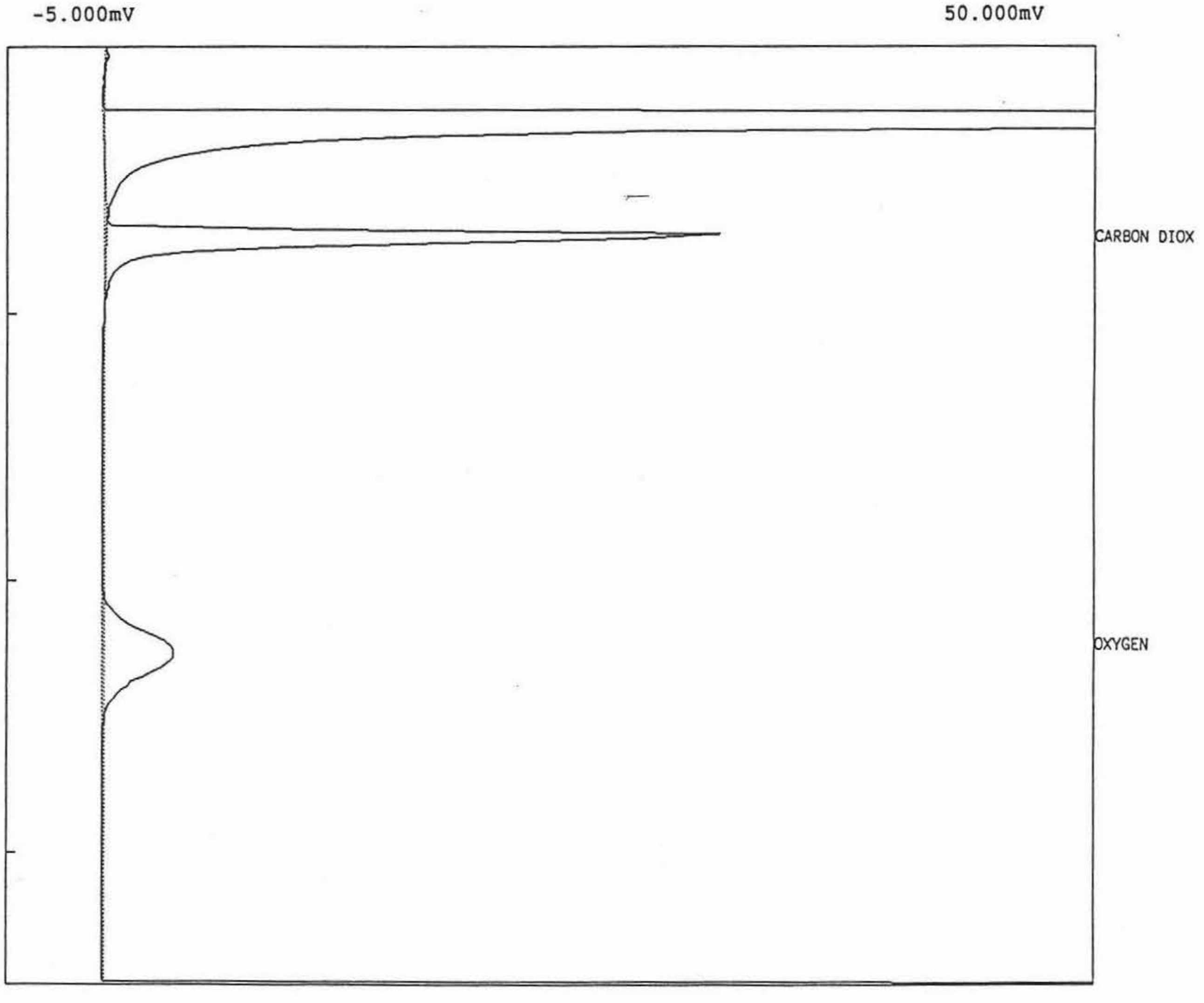
Carrier Gas:	Helium
Flow Rate:	75mL/min
Column Temperature:	30°C
Detector Temperature:	60°C
Detector Current:	High
Injection Volume:	1mL

The area under the peaks of the gases detected by the GC (Figure 3.1), was analyzed by a computer programme "Peak Master II" (SRI Instruments, Torrance, California, USA). The peak detection time intervals for the gases being analyzed are set out in Table 3.1.

Table 3.1 The Peak Detection Time Intervals for Carbon Dioxide and Oxygen.

Peak	Gas	Time (mins.)		Calibration
		Start	End	
1	Carbon dioxide	0.5	0.8	CTR1CO2.CAL
2	Oxygen	1.7	2.5	CTR1O2.CAL
3	Nitrogen	2.3	3.2	NITROGEN.CAL

Figure 3.1 Diagram of the Peaks Detected by the GC from a Sample Atmosphere.



Component	Retention	Height	Area	Area %	External	Units
	0.266	578.176	1005.24	87.31	N/A	
CARBON DIOXIDE	0.700	30.918	103.90	9.02	7.25 %	
OXYGEN	2.250	3.500	42.20	3.67	3.77 %	
3			1151.34	100.00	11.02	

The GC had to be calibrated to allow the computer programme to calculate the area under the peaks. This was done by preparing a standard curve using different amounts of pure gas (either carbon dioxide or oxygen). The GC was not standardised for nitrogen. The amounts of gas used and the resulting area under the peaks are set out in Table 3.2.

Table 3.2 The Data used to Generate a Standard Curve for Determining the Amounts of Carbon Dioxide and Oxygen from the Peak Area.

Injection No.	OXYGEN		CARBON DIOXIDE	
	Amount Injected mL	Area	Amount Injected mL	Area
1	0.10	3.37	0.05	0.78
2	2.00	22.0	0.10	1.14
3	4.36	49.0	2.00	28.8
4	8.72	97.0	5.00	71.0
5	13.08	147.0	10.0	144.0
6	17.44	203.0	15.0	215.0
7	21.80	260.0	20.0	293.0
8	43.20	520.0		

The GC was checked regularly by injecting 1mL of air to check the oxygen concentration (21%) and small volumes of carbon dioxide to check the carbon dioxide concentrations. Any variation outside the expected levels resulted in the GC being checked and recalibrated if this was required.

The standard error (SE) was calculated by injecting 1mL of air to provide the data for oxygen, as well as 0.1mL and 2mL of pure CO_2 for carbon dioxide data. The data and the average, sample standard deviation and SE are set out in Table 3.3.

Table 3.3 Standard Error Calculation for Gas Composition Analysis by the Gas Chromatograph

Injection No.	Oxygen % v/v[a]	1% CO_2 v/v	20% CO_2 v/v
1	21.69	0.81	20.82
2	21.94	0.94	20.82
3	21.46	0.87	20.56
4	21.82	0.81	
5	21.23	0.98	
6	21.79		
7	21.38		
Average	21.76	0.88	20.73
Sample Stdev	0.29	0.08	0.15
SE	0.11	0.04	0.09

Note: a) These oxygen data include the argon present in the atmosphere as well (about 0.9%), but still allows the SE to be calculated.

3.4 Taste Panels

The different taste panel methods used in this research are detailed in the appropriate chapter as each was tailored to the vegetable being tested.

CHAPTER 4

DETERMINATION OF THE PACKAGING PARAMETERS FOR CELERY STALKS WITHOUT RESPIRATION DATA

4.1 Introduction

The analysis of the packaging parameters in equations 4.1, 4.2 & 4.3 can be used to determine the design of polymeric packaging for produce where the respiration rate is not known. Mannapperuma and Singh (1990) used this technique in a storage study with cauliflower florets and this study on modified atmosphere packaging of celery stalks used the same method to determine the packaging parameters.

The polymer type was selected by comparing the recommended modified atmosphere with the polymer permeability ratio, β, which is the ratio of the permeability coefficient of the film to CO_2 and to O_2 (Mannapperuma and Singh (1990)).

$$x_1 = c_1 - \frac{R_1}{P_1}\phi \qquad (4.1)$$

$$x_2 = c_2 + \frac{R_2}{P_2}\phi \qquad (4.2)$$

where

$$\phi = \frac{Wb}{A} \qquad (4.3)$$

and

A	=	area of film (m^2)
b	=	thickness of the film (m)
c	=	gas concentrations in ambient (moles/m^3)
P	=	permeability coefficient (m^2/s)
R	=	respiration rate (moles/kg.s)
W	=	weight of produce in the package (kg)
x	=	gas concentrations in the package atmosphere (moles/m^3)

suffixes 1 and 2 denote oxygen and carbon dioxide respectively.

4.2 Procedure

4.2.1 Determination of Package Size and Produce Weight

The recommended modified atmosphere for the storage of celery was 2% - 4% O_2 and 3% - 5% CO_2 (Mannapperuma and Singh (1990), see Figure 4.1, Saltveit (1989) and Reyes (1989)). The permeability ratio, β, of the best film to use was determined using the slope of the line A-B passing through the region of the recommended atmosphere for celery. A permeability ratio, β, of between 4.2 and 7 is required, and polyethylene would fit this requirement, as is shown in Table 4.1.

Table 4.1 A Summary of Permeability Ratios for Low Density Polyethylene from Various References.

Literature	Permeability Ratio β
Combellick (1985)	3.8 - 5.6
Mannapperuma & Singh (1990)	5.0
Pauly (1989)	4.3

The respiration rate for celery stalks at this recommended modified atmosphere is not known and an experimental method, based on Mannapperuma and Singh (1990) was used to determine a suitable ϕ using equation 4.3. A number of packages with different values of ϕ were prepared by using the same bag size with different weights of celery.

As the cut surface of the celery goes brown due to enzymic activity after trimming, an additional wash treatment was used to minimize this activity, as suggested by Armstrong (1990). The celery was washed in a cold water bath (at 7°C) containing either

a) 1% ascorbic acid, 1% citric acid and 1% salt (acid wash), or

b) 200ppm sulphur dioxide (SO_2) by the addition of sodium metabisulphite.

Celery from the final wash was then packed in 100mm x 400mm x 60μm polyethylene bags with the following weights of celery stalks: 50g, 100g, 200g, 250g and 300g. The upper limit was determined by the maximum amount of celery able to be placed into the bag. These were stored in a chiller at 4-5°C.

The gas composition inside each bag was measured at regular intervals until it reached equilibrium. The results of this trial were used to determine the weight per package to be used for the storage trials.

Samples were evaluated for microbial counts (including the presence of *Listeria monocytogenes*) to determine the effectiveness of the chlorine wash and the effect of storage.

Samples of the two final wash options plus a control without treatment, were also packed in 300g packs to evaluate the anti-browning treatment by comparing the visual change in the cut surface colour. The samples were subjected to sensory evaluation using a triangle test method with 18 experienced panellists, comparing the treated samples with the control. The evaluation procedure form supplied to the panellists is set out in Figure 4.2.

Figure 4.1 Recommended Modified Atmospheres for Storage of Vegetables (from Mannapperuma and Singh (1990))

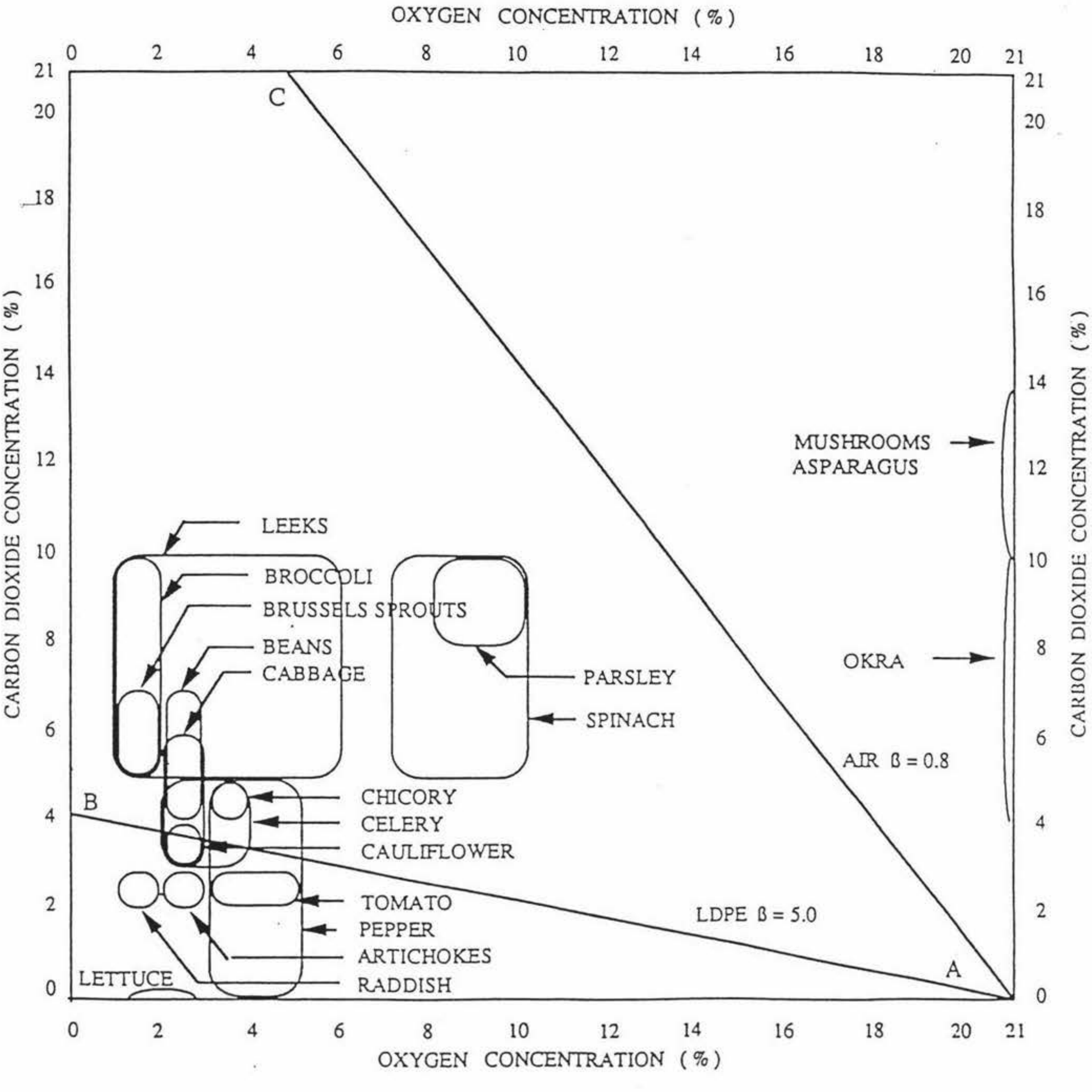


Figure 4.2 Triangle Taste Panel Procedures Form

CELERY TRIANGLE TASTE PANEL

EVALUATION PROCEDURE

1. Rinse your mouth thoroughly.

2. Taste test samples in the order presented.

3. Please evaluate each sample in the same manner, taking a similar bite size and chewing for roughly the same amount of time before swallowing.

4. Rinse mouth thoroughly after each sample. This is to remove flavour residue.

5. Be careful not to muddle samples.

6. Please comment on any differences, similarities, preferences and in general.

7. If you must re-taste samples, please use the same evaluation procedure as before.

4.2.2 Storage Trials for the Packaged Celery Stalks

Based on the first part of this research 300g of prepared celery stalks (with the "acid" anti-browning treatment) were packed in either air or flushed with a mixture of 4.5 -5% CO_2 and 3 - 4% O_2 in polyethylene bags (100mm x 400mm x 70µm) and stored at 5°C, 10°C, and 17°C. The celery at 10°C and 17°C was evaluated for softness and visible rot to determine an end-point. Further packs of the SO_2-treated celery stalks were packed in air and stored at 5°C. The packages of celery that started with an atmosphere of air and were stored at 5°C were sensory evaluated weekly for texture and flavour using the method detailed below. The flushed packages were sensory evaluated only at the end of the produce shelf life which was considered to be when the celery stalks were similar to the "old" calibration sample described below.

A panel of 10 experienced taste panelists selected from the Massey University campus used the following procedure to evaluate the celery. Selection of the panelists was based on their availability throughout the trial and whether they normally eat celery.

4.2.3 Taste Panel Procedures

The procedure used in the taste panel evaluation of the stored samples of celery was:

i) Two calibration samples were supplied for initial evaluation of celery flavour and celery crispness using a 7 point scale. These were to provide reference points. The celery flavour scale went from "old celery flavour (1)", to "fresh celery flavour (7)"; and the crispness being "soft (1)", to "crispy (7)". The calibration samples used were a very fresh sample of celery obtained directly from the grower and kept cool (these celery bunches were less than 24 hours from harvest) and an "old" sample purchased from a local supermarket and left at ambient temperature for 24 hours. The "old" sample has an estimated time from harvest to chilling for the taste panel of 48 hours at ambient temperatures. Several hours before the taste panel the old sample was chilled to prevent a temperature effect when the samples were tasted.

The panel then discussed the results for flavour and crispness of the two calibration samples and agreed on a common score as a reference score for the evaluation of the trial celery. The panel forms used are presented in Figures 4.3 and 4.4.

ii) The trial celery was diced into 30 mm lengths, given a three number code and presented to the panellists for evaluation using the 7 point scales for flavour and crispness. The results of this evaluation were averaged to provide a score for each trial sample.

gure 4.3 Celery Storage Trial Taste Panel Procedure Form

CELERY TASTE PANEL

EVALUATION PROCEDURE

1. Rinse your mouth thoroughly.
2. Taste first calibrating sample. Panel will agree on a flavour and crispness score for this product. (Note steps 5 and 6).
3. Taste second calibration sample. Panel will agree on scores. (Note steps 5 and 6).
4. Taste test samples in the order presented.
5. Please evaluate each sample in the same manner, taking a similar bite size and chewing for roughly the same amount of time before swallowing.
6. Rinse mouth thoroughly after each sample. This is to remove flavour residue.
7. Be careful not to muddle samples.
8. Please score celery based on the scales below. Please do not score using halves. A score is required for each sample.

A. CELERY FLAVOUR

1	2	3	4	5	6	7
Old celery flavour						Fresh celery flavour

B. CELERY CRISPNESS

1	2	3	4	5	6	7
Soft celery						Crispy celery

9. Please comment on any differences, similarities, preferences and in general.
10. If you must re-taste samples, please use the same evaluation procedure as before.
11. There will be a short discussion after everyone has finished evaluating the samples.

Figure 4.4 Celery Storage Trial Taste Panel Form

CELERY TASTE PANEL

Date: ....

Code: ....

Assessor: ....

You will be tasting two calibrating samples and then a number ( ) of other samples.

Please score samples based on the scale in the Evaluation Procedure.

Sample	Celery Flavour Score	Celery Crispness Score
Cal 1 (Fresh)		
Cal 2 (Older)		
Test No.		
Test No.		
Test No.		
Test No.		

Comments:

4.3 Results

4.3.1 Determination of pack size and celery weight

The gas composition of the packs with different weights of celery are set out in Table 4.2, with the resulting graphs in Figures 4.5 and 4.6.

Table 4.2 Gas Composition of the Headspace of Packages Containing Different Weights of Celery.

Time (days)	CELERY WEIGHT									
	50g		100g		200g		250g		300g	
	CO_2%	O_2%	CO_2 %	O_2%	CO_2%	O_2%	CO_2%	O_2%	CO_2%	O_2%
0	0.03	21.00	0.03	21.00	0.03	21.00	0.03	21.00	0.03	21.00
3	1.86	17.86	1.89	18.09	3.46	12.86	3.13	14.35	4.69	9.11
4	1.91	17.35	1.62	19.43	3.82	10.84	3.54	12.47	4.82	6.14
5	1.55	16.91	0.32	21.46	3.57	9.95	3.47	11.35	4.41	5.29
6	1.48	17.21	0.85	21.36	3.59	8.90	3.66	9.77	4.49	4.41
7	1.22	16.65	0.95	18.11	3.24	10.13	4.01	8.86	4.90	3.64
11	0.89	17.37	1.59	17.30	2.86	10.95	3.59	6.70	3.90	2.73
12	0.85	16.91	1.41	17.74	2.43	10.36	3.43	6.18	3.81	2.64
13	0.89	17.36	1.29	18.88	2.73	10.68	3.35	5.95	3.72	2.81
14	0.72	17.52	1.30	18.94	2.57	9.87	3.50	5.05	4.11	2.84
17	0.79	16.92	1.18	19.63	2.42	8.31	4.19	4.02	3.71	2.55
18	0.94	18.26	1.03	20.24	2.34	7.93	4.18	3.24	4.17	2.53
19	0.77	16.73	1.20	20.24	2.72	7.41	4.38	2.64	4.19	2.19
20	0.92	16.97	1.24	19.70	2.77	7.18	3.79	2.56	4.11	2.12
21	0.88	16.71	2.02	19.30	2.46	7.01	4.14	2.52	4.16	2.07

Note: All packs started with a normal atmosphere gas composition.

The graph of the gas composition data for days 7 and 11, is set out in Figure 4.5. This graph shows increasing CO_2 and decreasing O_2 concentrations inside the package with increased weight of produce. According to Mannapperuma and Singh (1990) the increasing weight should produce a straight line. The celery data produced a reasonable straight line except for the 50g and 100g weights. The non-linear nature of the line at the lower weights could be due to the celery used. Whole celery stalks were used with minimal trimming, thus the smaller whiter central stalks were used in the 50g bag and these may have had higher respiration rates than the green outer stalks used for the 100g to 300g weights. The gas composition of the 300g pack was within the recommended modified atmosphere by day 7 but continued to change until day 11, when th gas composition appeared to reach an equilibrium and remained reasonable constant for the next 10 days (Figure 4.6).

Figure 4.5 Gas Composition of the Packages Containing Different Weights of Celery at Day 7 and 11.

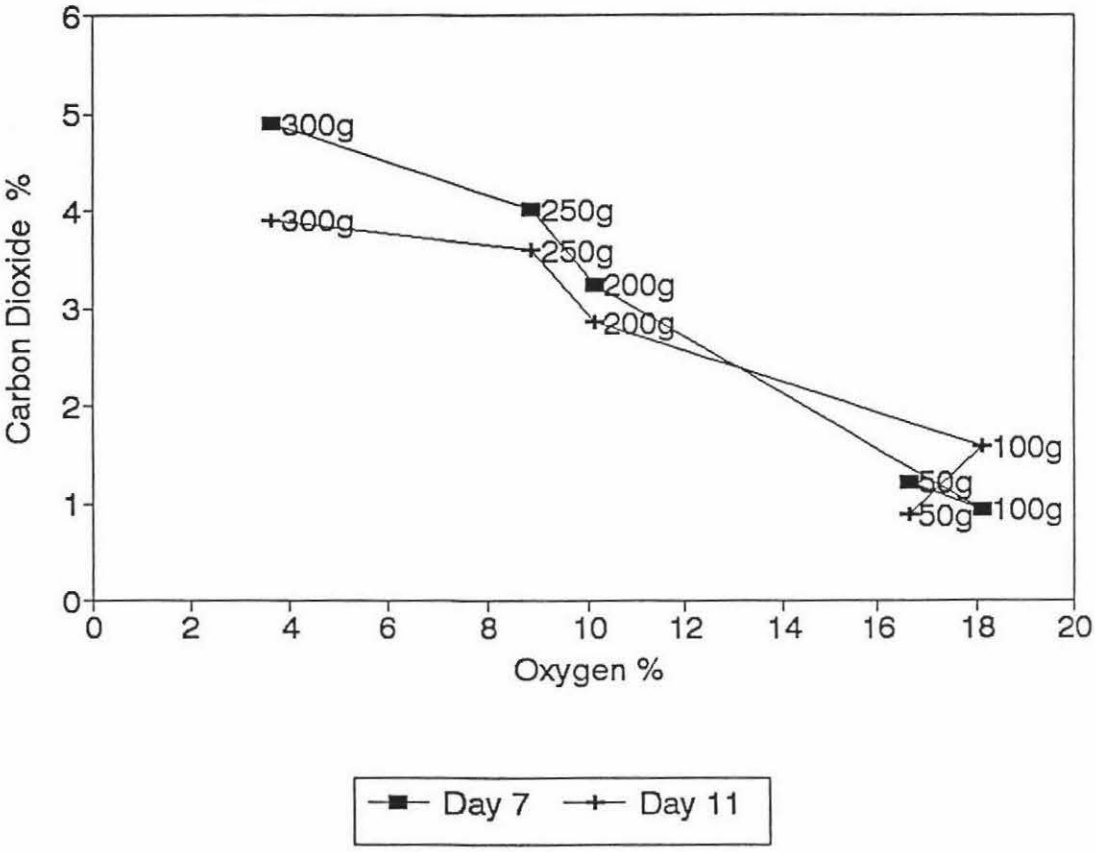
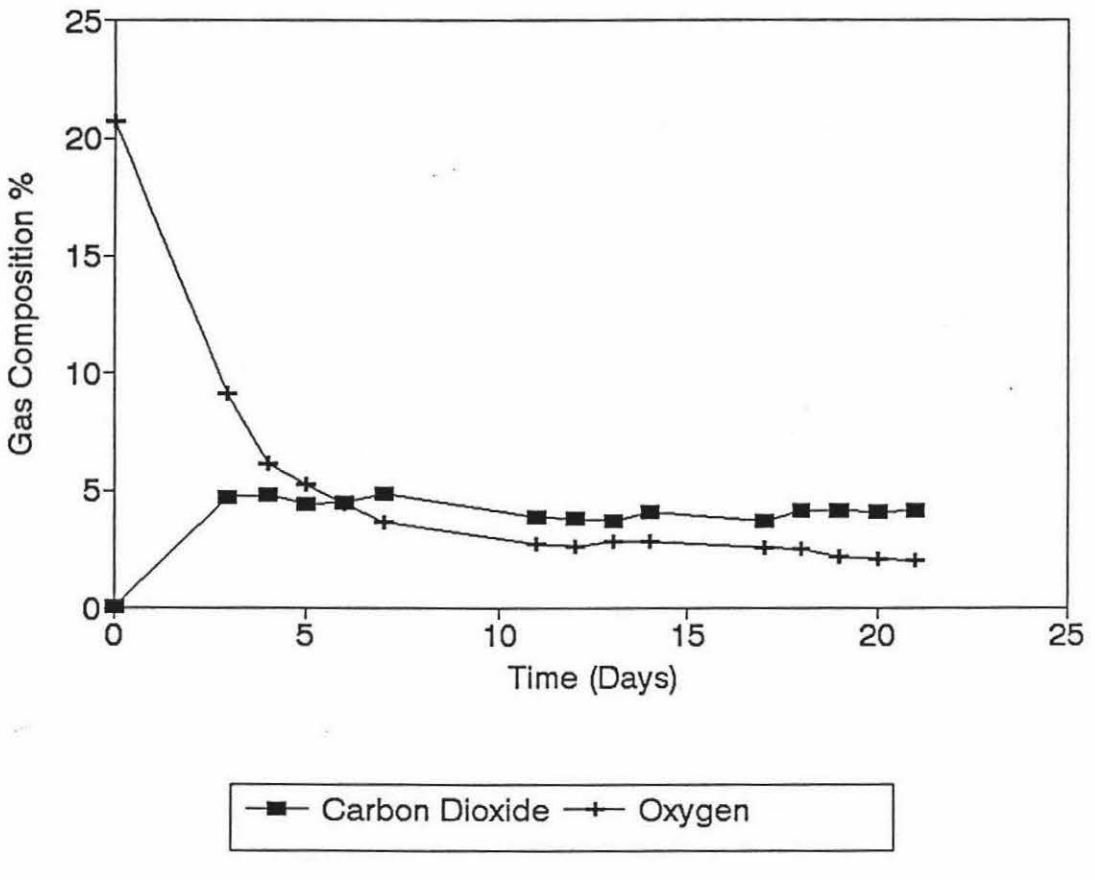


Figure 4.6 Gas Composition of the 300g Package of Celery over the Storage Trial Period.



From Figure 4.5 and 4.6 it can be seen that the equilibrium gas composition of the 300g pack is within the recommended MAP gas composition (2-4% O_2 ; 3-5% CO_2). This weight of celery stalks was used for the storage trials. Note: 300g of celery was close to the maximum quantity the package could hold with the large celery stalks being a very tight fit.

The sensory and visual evaluation of the two anti-browning treatments are detailed below with the results of the triangle test sensory evaluation of the two treatments for browning prevention set out in Table 4.3

Table 4.3 Results of the Triangle Sensory Evaluation of the Anti-Browning Treatments

Comparison	Number of Panellists	Number of Correct Answers	Level of Significance of Difference
SO_2 vs Control	9	7	1%
"Acids" vs Control	9	7	5%

The sulphur dioxide treatment resulted in less browning of the cut surface than the "acid" treatment or the control. The "acid" treatment was not only less effective than the SO_2 treatment but it was observed on chopping the celery for the taste panels that the freshly cut ends rapidly became brown even when compared to the control product. Both treatments changed the flavour of the celery (Table 4.3), with the difference between fresh celery and the SO_2 treated product being highly significant 99% and the difference between the control and the "acid" wash significant at 95% level. The SO_2 treatment was the most effective treatment for reducing the browning of the cut surfaces, but did result in a noticeable change in the flavour of the celery stalks.

4.3.2 <u>Storage Trials on the 300g Pack of Celery</u>

The storage trial sensory evaluation results for celery stored at 4°C - 5°C are set out in Table 4.4 with the microbiological results in Table 4.5.

Table 4.4 Results of the Sensory Evaluation of 300g packs of Celery Stalks Stored at 4°C - 5°C and Packed Air Atmosphere

Time Days	Calibration 1		Calibration 2		SO_2 Wash		"Acid" Wash	
	Flavour	Crisp	Flavour	Crisp	Flavour	Crisp	Flavour	Crisp
7	5	5.5	2.5	3.0	4.4	3.7	4.3	5.4
14	5.0	6.0	2.0	4.0	3.2	4.8	4.3	5.8
21	5.5	6.0	3.0	3.0	3.3	5.0	3.8	5.8
28	6.0	7.0	2.0	3.0			3.3	5.0
							3.2[a]	4.5[a]

Note a) These figures are for celery stalks packaged in bags flushed with 3% O_2 and 4% CO_2.

The samples at 28 days (4 weeks) were still crisp but some panellists commented about off notes present in the flavour. The stored celery stalks were almost as crisp as the fresh sample (Calibration 1) during the first 21 days of the trial, and crisper than the old samples (Calibration 2). The crispness of the stored samples noticeably decreased after 21 days. The flavour of the stored samples were not as good as the fresh sample at any stage during the storage trial, but they were better than the flavour of the old sample. Samples supplied to the client, a celery grower, for their evaluation were deemed satisfactory, with any changes in flavour being noted as due to the different harvest time of the celery. Discussion with the panellists as well as an analysis of the panel results indicated an acceptable shelf life of 14 days (2 weeks), which could be extended to 21 days. Panellist comments on the stored celery after 28 days included; "stringy", "tainted flavour - made assessment of 'celery' flavour difficult", "crunchy but lacked fresh flavour", "tasted old, musty, stale flavour but still crispy", "old dry taste", and "nasty and slightly off taste".

The microbiological results are detailed in Table 4.5; they show the success of the washing sequences in reducing the bacterial numbers from 750,000 colonies per cm^2 to 260 colonies per cm^2.

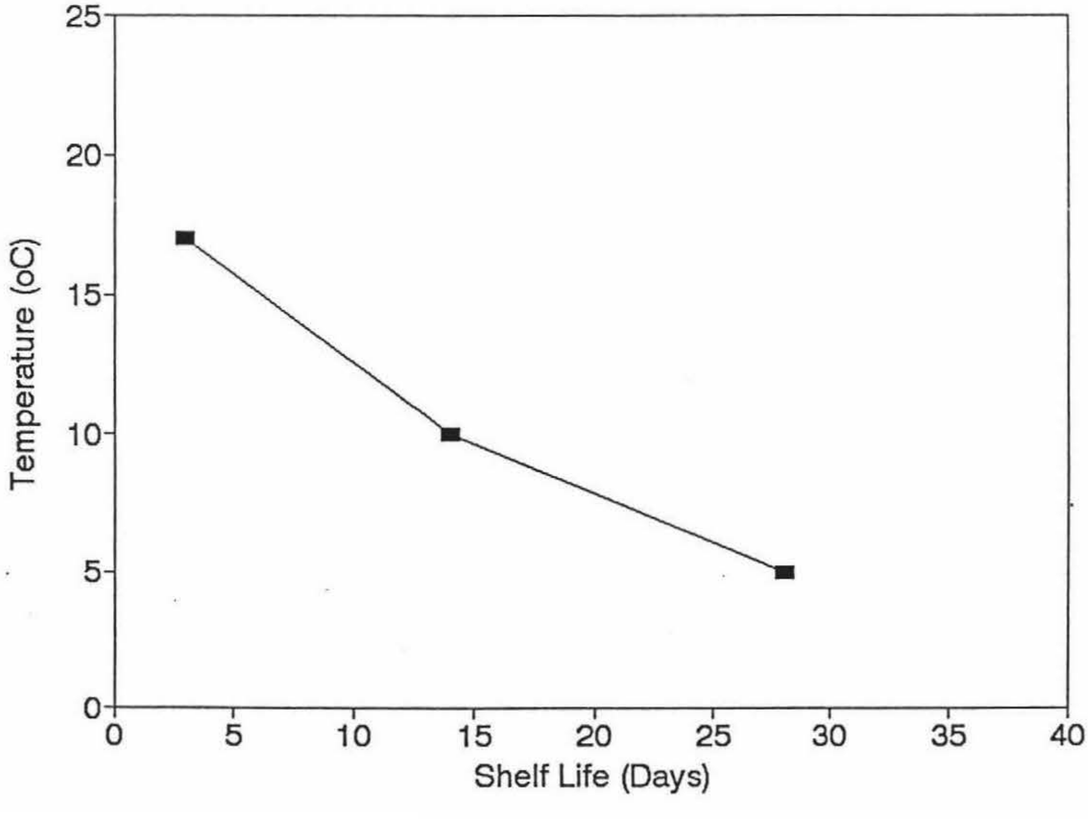
Table 4.5 Microbiological Results of Celery Stalks Before and After Washing and After 28 Days of Storage at 4°C - 5°C Packed in Air Atmosphere.

	CPU (Colonies per 1 cm^2)	Presence of Listeria
Pre washed celery	7.5×10^5	nil
Post wash celery	2.6×10^2	nil
28 Days storage		
Flushed	2.1×10^5	nil
Atmosphere	1.6×10^4	nil

There was a slow increase in bacterial numbers such that by the end of 28 days storage the numbers were still lower than the initial levels found on the unwashed celery. No *Listeria monocytogenes* were detected. This is important as this bacterium is an important cause of food poisoning and can survive and grow at 3°C to 5°C (normal chiller temperatures).

The shelf life obtained at the three temperatures used for the storage trial is shown in Figure 4.7. The celery stored at 17°C and 5°C were evaluated by the taste panel, with the product stored at 17°C being the "old" (calibration 2) sample. The end point of the celery stored at 10 °C was determined by visible wilting and very soft texture. At the end point for the celery stored at 5°C, the taste panel results show the celery looked acceptable and was still crisp, but had lost some celery flavour. The product stored at 17°C had both a soft texture and poor flavour after two days. The graph (Figure 4.7) should only be used as an indicator of trends for storing celery as the 10°C stored product was not evaluated using the taste panel. A more suitable storage trial would involve either evaluating the product flavour, which could be difficult at higher temperatures while maintaining panel safety, or measuring the product texture using the Instron Texture instrument. The graph (Figure 4.7) does illustrate, by extending the line out from 28 days, that the shelf-life of the packaged celery could be further increased by the use of a lower temperature of 1°C to 2°C, which is supported in the literature were the storage temperature for celery is given as 0°C to 5°C (Reyes (1989), Saltveit (1989)). Whether or not this would be commercially feasible would require further investigations that are not part of this study.

Figure 4.7 Shelf-Life of the Modified Atmosphere Packaged Celery Stalks Stored at 17°C, 10°C and 5°C.



4.4 Discussion

The method used for determining the packaging material and package dimensions for the celery stalks provided a successful package that was able to maintain the desired atmosphere for over 28 days, as can be seen in Figures 4.6 & 4.7. It was assumed that the recommended storage conditions set out by Mannapperuma and Singh (1990), Saltveit (1989) and Reyes (1989) would be appropriate for the locally grown celery product. The package realistically should be regularly evaluated over the whole of the growing season of the celery to ensure that any changes that may occur do not affect the package design. Some of the changes that may occur during the growing season include:

a) different varieties of celery, and

b) effect of the growing conditions as the growing season starts in spring and continues through to autumn as mature celery is harvested throughout this period.

Time did not permit this investigation, as the client required the pack design urgently. The major disadvantage of this method of determining the package design is the requirement for published recommended storage atmospheres to be available. These data are becoming increasingly available for many vegetables, but not for vegetables that have been partially processed. This method with the available recommended storage atmospheres would be very suitable for commercial application as can be seen from the successful application to celery stalks. A factory for processing the packaged celery is being set up for commercial evaluation.

4.5 Summary

The development of the modified atmosphere package for the celery stalks followed the method recommended by Mannapperuma and Singh (1990) and it worked for polyethylene bags. The method, however, has a number of limitations including:

a) The requirement to arbitrarily select the thickness of the polymer film. The selection of the correct polymer film thickness could involve multiple trials. The polymer thickness chosen for packaging the celery stalks only just meets the requirements of maintaining the required modified atmosphere. Also, the 300g of celery stalks is a tight fit in the selected bag size.

b) There is still a large element of trial and error in the method, and if other polymers were to be evaluated the whole experiment would have to be rerun.

c) The availability in the literature of the recommended modified atmospheres and storage temperatures. This information is not readily available for minimally processed vegetables.

However, once the correct weight of produce is found for the selected polymer thickness and bag dimensions, equation 4.3 can be used to design other bag/weight combinations. Using equation 4.3, ϕ for the celery stalks is 2.625×10^{-4}, and using this value back in equation 4.3 the following bags will maintain the required modified atmosphere:

300g celery stalks in a 400mm x 171mm x 120μm polyethylene bag
400g celery stalks in a 400mm x 133mm x 70μm polyethylene bag

The flexibility which the equation allows the commercial grower is useful for quickly designing different packages.

The application of experimental design techniques to the recommended method of Mannapperuma and Singh (1990) recommended method would result in a more rapid selection of the appropriate package for the produce.

CHAPTER 5

DESIGNING A MODIFIED ATMOSPHERE PACKAGE FOR PEELED GARLIC

5.1 Aim

To develop a modified atmosphere package for peeled garlic using the mathematical model developed by Mannapperuma and Singh (1990), and respiration data determined by a Response Surface Methodology experimental design.

5.2 Procedure

5.2.1 Determination of the optimum combination of oxygen and carbon dioxide for the modified atmosphere.

Response Surface Methodology (RSM) was used to design and analyze this part of the experiment. The base parameters used for this experiment are set out in Table 5.1 with the experimental design developed by the RSM programme in Table 5.2.

Table 5.1 The parameters used to design the experiment.

Parameters	Levels				
Oxygen	3%	5%	10%	15%	18%
Carbon dioxide	3%	5%	10%	15%	18%

Table 5.2 The experimental design developed by the RSM programme

Seq. No.	Plan No.	% Oxygen	% Carbon Dioxide
1	2	15	5
2	10	10	10
3	8	10	17
4	4	15	15
5	9	10	10
6	5	3	10
7	3	5	15
8	6	17	10
9	13	10	10
10	11	10	10
11	1	5	5
12	7	10	3
13	12	10	10

The responses to be measured were the changes in the composition of the atmosphere from which the Oxygen Respiration Rate and Carbon Dioxide Respiration Rate were calculated to be entered into the programme as the response results.

The respiration rates were determined using a method similar to that used by Jurin & Karel (1963). 150g of peeled garlic was weighed into an Agee preserving jar, which was then filled with the required gas mixture. The gas mixture was generated by an LNI MGA-I $CO_2/O_2/N_2$ Mixer, and the composition of the gas from the mixer checked using the Gas Chromatograph (GC). The Agee jar was flushed with the gas mix before sealing and the gas composition in the jar checked using the GC and recorded.
The jars were stored at 5°C for 24 hours after which time the gas composition was measured again. The following data were required to calculate the respiration rates of the peeled garlic:

Time - start and finish times;
Weight - accurate weight of peeled garlic in the jar;
Free Volume in the Jar - measured by water displacement, i.e. the jars were filled with water and the increased weight recorded as free volume;
Gas composition in the jar - at the start and end of the time period.

The respiration rates were then calculated using the equations developed by Mannapperuma & Singh (1990), for respiration rate measurement in a closed system. Equation 5.1 was used to calculate the oxygen respiration rate, and Equation 5.2 used to calculate the carbon dioxide respiration rate.

$$R_1 = \frac{V}{W}\frac{dx_1}{dt} \tag{5.1}$$

$$R_2 = \frac{V}{W}\frac{dx_2}{dt} \tag{5.2}$$

Where:

R = respiration rate (moles/kg.s)
W = weight of produce in the package (kg)
V = volume (m^3)
x = gas concentrations in the package atmosphere (moles/m^3)
dt = time interval between gas atmosphere measurements (sec)
dx = change in gas concentrations over time interval dt (moles/m^3)
suffixes 1 and 2 denote oxygen and carbon dioxide respectively.

These calculations were set up on a spreadsheet (Quattro Pro) and the data entered (see Appendix 1 for a copy of the spreadsheet used).

The calculated respiration rates were used to identify the region of lowest respiration for the peeled garlic.

5.2.2 Pack Size and Type Determination.

The oxygen and carbon dioxide respiration rates corresponding to the region of least respiration were used to calculate the bag dimensions, polymer type, film thickness, and produce weight using equations 5.3 & 5.4. The polymer type was selected by using the recommended atmospheric composition, x_1, and x_2, to determine the required ratio of the permeability coefficient of the film to CO_2 and O_2. This ratio is denoted by the symbol β.

$$x_1 = c_1 - \frac{R_1}{P_1}\phi \tag{5.3}$$

$$x_2 = c_2 + \frac{R_2}{P_2}\phi \tag{5.4}$$

where

$$\phi = \frac{Wb}{A} \tag{5.5}$$

and

A = area of film (m^2)
β = ratio of permeability coefficients of film to CO_2 and O_2
b = thickness of the film (m)
c = gas concentrations in atmosphere (moles/m^3)
P = permeability coefficient (m^2/s)
R = respiration rate (moles/kg.s)
W = weight of produce in the package (kg)
x = gas concentrations in the package atmosphere (moles/m^3)
suffixes 1 and 2 denote oxygen and carbon dioxide.

The designed bags were then evaluated during shelf-life trials at 5°C, 10°C and 17°C.

5.2.3 Shelf-life Trials

The pre-preparation of the garlic before packaging is critical to the success of the shelf-life trials as previous experience had demonstrated the importance of keeping bacterial numbers to a minimum (unpublished work - see Chapter 6). The preparation procedure outlined in Chapter 3, Section 3.1.1 for washing celery was used to prepare the peeled garlic.

The calculated weight of washed, peeled garlic was packed into the required bags (see Table 5.8, calculated during the research outlined in Section 5.2.2), flushed with the required gas atmosphere (Table 5.8), sealed and placed into storage at 5°C, 10°C and 17°C. The bags were examined weekly for visible signs of mould growth and bacterial soft rot, and samples evaluated by expert garlic tasters near the end of the shelf-life for flavour and texture.

5.2.4 Taste Panel Evaluation

The samples to be evaluated for flavour and texture were placed in a polystyrene box with a small amount of ice and sent by courier to a Garlic Grower Cooperative in Blenheim. The Cooperative's expert garlic tasters evaluated the peeled garlic using the following method.

5.2.4.1 Sample Preparation

Samples were prepared just before the taste panel commenced by;

(a) Removing plain cottage cheese from the refrigerator 2 hours beforehand

(b) Weighing out 12.5g of garlic cloves
(c) Mincing the garlic using a garlic crusher
(d) Adding the crushed garlic to 250 g cottage cheese and mixing well
(e) Dividing the mixture into portions for each taster

The person preparing the samples was asked not to participate in the tasting, but to run the panel, including preparing and delivering samples to the panel.

5.2.4.2 Panel Set Up

The panellists were provided with:

(a) A glass of water - held in jug for 10 minutes before taste panel
(b) A questionnaire, evaluation procedure and pen
(c) 50 - 100g natural cottage cheese as a palate cleaner
(d) A plastic teaspoon for each sample

5.3.4.3 Taste Panel

The following instructions were provided for the operation of the panel:

(a) 5 - 10 people to taste garlic
(b) Serve control sample first (this is the one made from fresh raw garlic)
(c) Then serve the other samples
(d) Ensure that the code number assigned stays with the correct sample
(e) Ensure that panellists do not talk during session so that their opinions will be independent
(f) The panel should be held in an odour free environment with no

distractions
(g) Ask the panel not to smoke, drink or eat for 20 minutes before the panel
(h) A sweet reward given at the end of the session is often good for panellist motivation
(i) A fresh control sample must be made for each panel session

Each panellist was provided with the procedure form (Figure 5.1) and the evaluation form (Figure 5.2).

5.2.5 Microbial Evaluation

The effectiveness of the preparation wash method at reducing the microflora of the peeled garlic was evaluated by doing Total Plate Counts of samples taken from cloves before and after washing.

Samples from the storage trials were also submitted to a laboratory for microbial Total Plate Counts. Total Plate Count (TPC) is a basic procedure to determine the number of bacteria present under normal aerobic conditions. This involves mixing a weighed sample of the garlic with sterile peptone water, followed by a series of dilutions. The dilutions are then plated out onto nutrient agar and incubated at 35°C for 2 - 3 days after which the colonies are counted.

5.3 Results

5.3.1 Determination of the optimum combination of oxygen and carbon dioxide for the modified atmosphere.

The data from the RSM experiment are presented as follows:

a) Table 5.3 contains the time between gas composition analyses and the actual concentrations of the two gases measured. The data is from duplicate samples except for jar 9B which leaked and was not repeated.

b) Table 5.4 contains the weight of garlic and the measured free volume in each jar.

The carbon dioxide and oxygen values are the actual measured values and the jar number corresponds to the Sequence Number in Table 5.2. The data were then used to calculate the O_2 and CO_2 respiration rates of the garlic in each jar, using equations 5.1 and 5.2; the results are presented in Table 5.5. The Spreadsheet showing the methods used to calculate the respiration rates is shown in Appendix 1.

GARLIC TASTE PANEL

Evaluation Procedure:

1. Rinse your mouth thoroughly.

2. Taste samples in the order presented.
(The control will be the first sample in each session).

3. Please evaluate each sample in the same manner, taking a similar spoonful and chewing for roughly the same amount of time before swallowing.

4. Rinse mouth thoroughly after each sample, then eat a spoonful of the plain cottage cheese provided. This is to remove flavour residue.

5. Be careful not to muddle samples.

6. Please score garlic based on the scale below. Please do not score using halves. A score is required for each sample.

   Scale: Garlic Flavour

0	1	2	3	4	5
No Garlic Flavour					Strong Garlic Flavour

7. Please comment on any differences, similarities, preferences and in general.

8. If you must re-taste samples please use the same evaluation procedure as before.

Figure 5.1 The Procedure Form Used for the Taste Panel Evaluation of the Packaged Peeled Garlic

GARLIC TASTE PANEL

Date: ______________________________

Code: ______________________________

Assessor: ______________________________

You will be tasting a control sample and then a number (______) of other samples. Please score samples based on the scale in the Evaluation Procedure.

	Sample			
	Control			
Garlic Flavour Score	5			

Comments:

Figure 5.2 The Taste Panel Form Used for the Garlic Taste Evaluation

Table 5.3 Time and Gas Composition Data for Peeled Garlic from the Response Surface Methodology experiment

Jar No.	Time In (hours)	Time Out (hours)	Carbon Dioxide		Oxygen	
			Initial % V/V	Final % V/V	Initial % V/V	Final % V/V
1A	16.37	38.37	4.9	7.9	14.9	7.6
1B	16.46	38.91	4.7	8.4	14.6	7.5
2A	17.27	39.62	9.1	11.6	10.5	5.2
2B	17.34	39.86	8.7	10.9	10.2	6.2
3A	11.37	37.19	17.0	17.4	10.0	4.0
3B	11.48	37.28	17.8	18.4	9.3	2.4
4A	11.79	37.36	14.5	14.6	14.7	8.5
4B	11.86	37.43	14.0	15.8	14.9	7.4
5A	17.41	39.98	8.8	10.9	11.0	4.8
5B	17.47	40.08	8.8	10.8	10.5	4.8
6A	13.10	37.51	14.5	14.6	3.2	0.1
6B	13.20	37.59	9.2	9.9	3.6	1.3
7A	16.91	37.70	14.7	15.4	5.7	0.1
7B	16.98	37.81	14.4	16.1	5.7	1.3
8A	13.98	37.84	9.0	12.0	17.3	9.4
8B	14.12	37.91	8.4	11.2	17.2	10.7
9A	17.55	40.39	8.4	11.3	10.5	4.6
9B	Leaked					
10A	16.77	33.34	9.7	11.0	9.6	4.9
10B	16.85	33.49	9.2	10.6	9.7	4.7
11A	14.56	38.00	4.9	7.8	5.9	4.7
11B	14.68	38.08	4.6	9.6	5.6	0.9
12A	16.62	38.04	2.6	7.4	10.7	1.5
12B	16.74	38.13	2.6	6.6	10.7	4.4
13A	16.94	33.57	9.8	11.6	9.5	4.4
13B	17.02	40.08	9.0	10.3	10.0	6.1

Table 5.4 Weight of Peeled Garlic per Jar for the RSM Experiment.

Jar No.	Weight (grams)
1A	150.7
1B	150.7
2A	150.5
2B	150.1
3A	150.7
3B	150.1
4A	150.2
4B	150.0
5A	150.2
5B	150.0
6A	150.3
6B	150.9
7A	150.8
7B	150.1
8A	150.1
8B	150.2
9A	150.7
9B	150.1
10A	150.2
10B	150.8
11A	150.1
11B	150.4
12A	150.4
12B	150.5
13A	150.7
13B	150.2

Table 5.5 Calculated Carbon Dioxide and Oxygen Respiration Rates for Peeled Garlic.

Jar No.	Calculated Respiration Rates				
	Carbon Dioxide		Oxygen		Respiration Quotient
	(moles.kg^{-1}.s^{-1}) (x10^{-8})	(mg.kg^{-1}.hr^{-1})	(moles.kg^{-1}.s^{-1}) (x10^{-8})	(mg.kg^{-1}.hr^{-1})	(CO_2/O_2)
1A	9.3	14.7	22.2	25.6	0.42
1B	13.3	21.0	25.2	29.0	0.53
2A	7.8	12.3	16.6	19.1	0.47
2B	7.0	11.1	12.7	14.6	0.55
3A	1.2	2.0	16.3	18.8	0.07
3B	1.6	2.5	19.0	21.8	0.08
4A	0.4	0.1	17.2	19.8	0.02
4B	5.1	8.1	20.7	23.8	0.26
5A	6.7	10.5	19.3	22.2	0.35
5B	6.2	9.8	17.5	20.2	0.35
6A	4.9	7.7	9.0	10.4	0.54
6B	1.9	3.1	6.6	7.7	0.29
7A	2.2	3.5	16.1	18.6	0.14
7B	5.7	9.0	14.9	17.1	0.38
8A	9.0	14.2	23.4	27.0	0.39
8B	8.2	13.0	19.3	22.2	0.42
9A	9.0	14.2	18.0	20.8	0.50
9B	Leaked				
10A	5.6	8.9	19.9	22.9	0.28
10B	5.6	8.9	21.2	24.4	0.26
11A	8.0	12.6	3.3	3.8	2.42
11B	13.5	21.4	12.4	14.3	1.09
12A	15.7	24.9	30.2	34.8	0.52
12B	13.1	20.8	20.4	23.6	0.64
13A	7.3	11.5	21.6	24.9	0.34
13B	5.4	8.5	16.4	18.9	0.33

5.3.2 Package Design

The respiration data from Table 5.5 and the free volume of the jars (870mL were then fed into the Response Surface Methodology programme for analysis. All the numbers in the topographical maps generated by the RSM programme are smaller by a factor of 10^{-8}, since the actual data in Table 5.5 were used. The RSM programme could not handle exponential figures. The data was corrected in Figures 5.1 and 5.2.

5.3.2.1 Determination of the Optimum Oxygen Respiration Rate for Package Design

The RSM calculated the Taylor Expansion equation (5.6) for the oxygen respiration rate, from which the topographical map (Figure 5.1) has been developed.

$$Y_i = 18.163 + 4.688x_1 - 0.962x_2 - 2.256x_1^2 + 1.131x_2^2 - 2.865x_1x_2 \quad (5.6)$$

where

Y_1 is the oxygen respiration rate
x_1 is % oxygen
x_2 is % carbon dioxide

The RSM programme calculated the confidence levels of this equation as follows;

Standard Error = 2.80

Multiple Correlation Coefficient = 0.909

82.65% of the variation is explained by the Model

57.2% by First Order (Linear) effects
15.1% by Second Order (Quadratic) effects
10.4% by Interaction effects

6.2% of the variation is experimental error
11.2% of the variation is unexplained by either the Model or the Experimental error.

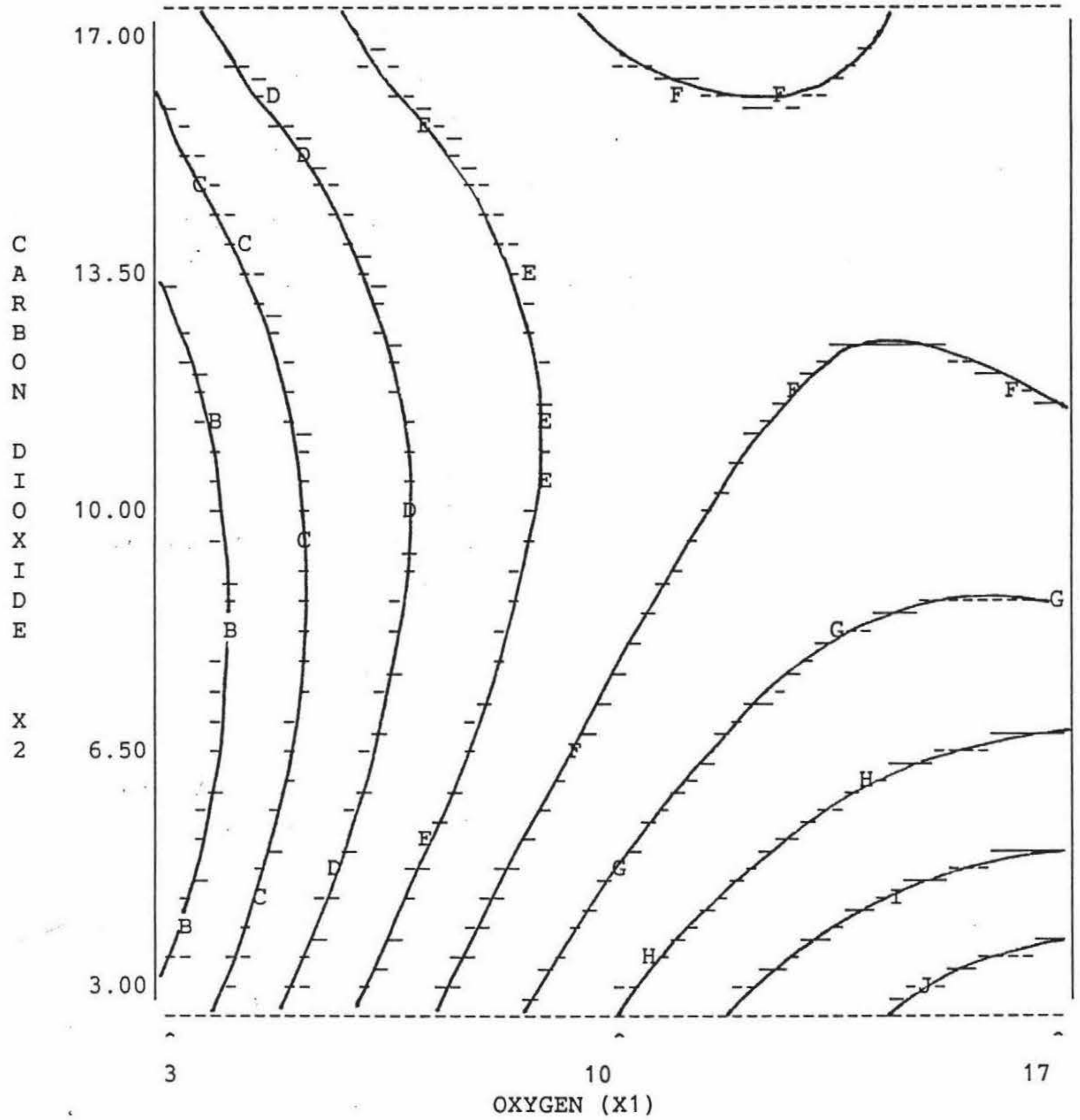
Analysis of variance indicates the First Order reaction is significant at 99% confidence and the Interaction at 90% confidence. A full printout from the RSM programme including the above data can be seen in Appendix 2.

RSM developed a topographical map of oxygen respiration rates as shown in Figure 5.1, where the lines on the graph represent the oxygen respiration rates from 1.2×10^{-7} moles.kg^{-1}.s^{-1} (line B) to 32.0×10^{-8} moles.kg^{-1}.s^{-1} (line J). The region between the lines on the map represent changing respiration rates that can be accurately determined by using equation 5.6. The map shows a steadily decreasing oxygen respiration rate as would be expected with a decreasing oxygen percentage in the atmosphere, similar to that reported by other researchers (Cameron, 1989; Henig & Gilbert, 1975). At high oxygen levels in the atmosphere (10% - 17%), increasing levels of CO_2 caused a decrease in the oxygen respiration rate, while at lower O_2 levels (3% - 10%) increasing CO_2 percent in the atmosphere had little effect on the oxygen respiration rate.

The region of lowest oxygen respiration rate is around 3% O_2 (line B) according to this map. As this experiment did not explore the region below 3% O_2, another experiment will be required to fully map this region. The SURFER computer programme can be used to produce topographical graphs over a wider area (see graphs in Appendix 3), but this can lead to errors from extrapolating data outside of the experimental region. Based on Figure 5.3 the recommended modified atmosphere for peeled garlic would be in the region of lowest oxygen respiration rate: below line 'B' in Figure 5.3 at 7.5 x 10^{-7} moles.kg^{-1}.s^{-1} and about 3% oxygen and 3% to 10% carbon dioxide.

igure 5.3 RSM Developed Topographical Map of the Oxygen Respiration Rate at Different Oxygen and Carbon Dioxide Levels for Peeled Garlic.

X-axis is percent oxygen v/v and the Y-axis is percent carbon dioxide v/v)



Where lines on the face of the map are the oxygen respiration rate in moles.$kg^{-1}.s^{-1}$ with following values:

A	=	not shown	G	=	2.5×10^{-7}
B	=	1.2×10^{-7}	H	=	2.7×10^{-7}
C	=	1.4×10^{-7}	I	=	3.0×10^{-7}
D	=	1.7×10^{-7}	J	=	3.2×10^{-7}
E	=	1.9×10^{-7}	K	=	not shown
F	=	2.2×10^{-7}	L	=	not shown

5.3.2.2 Determination of the Optimum Carbon Dioxide Respiration Rate for Package Design

The RSM calculated the Taylor Expansion equation (5.7) for the carbon dioxide respiration rate, from which the topographical map (Figure 5.2) has been developed.

$$Y_{11} = 6.945+0.729X_1-4.099X_2-0.489X_1^2+0.546X_2^2-0.212X_1X_2 \qquad (5.7)$$

where

Y_{11} is the carbon dioxide respiration rate
x_1 is % oxygen
x_2 is % carbon dioxide

The RSM programme calculated the confidence levels of this equation as follows;

Standard Error = 1.61

Multiple Correlation Coefficient = 0.942

88.69% of the variation is explained by the Model

86.0% by First Order (Linear) effects
2.58% by Second Order (Quadratic) effects
0.11% by Interaction effects

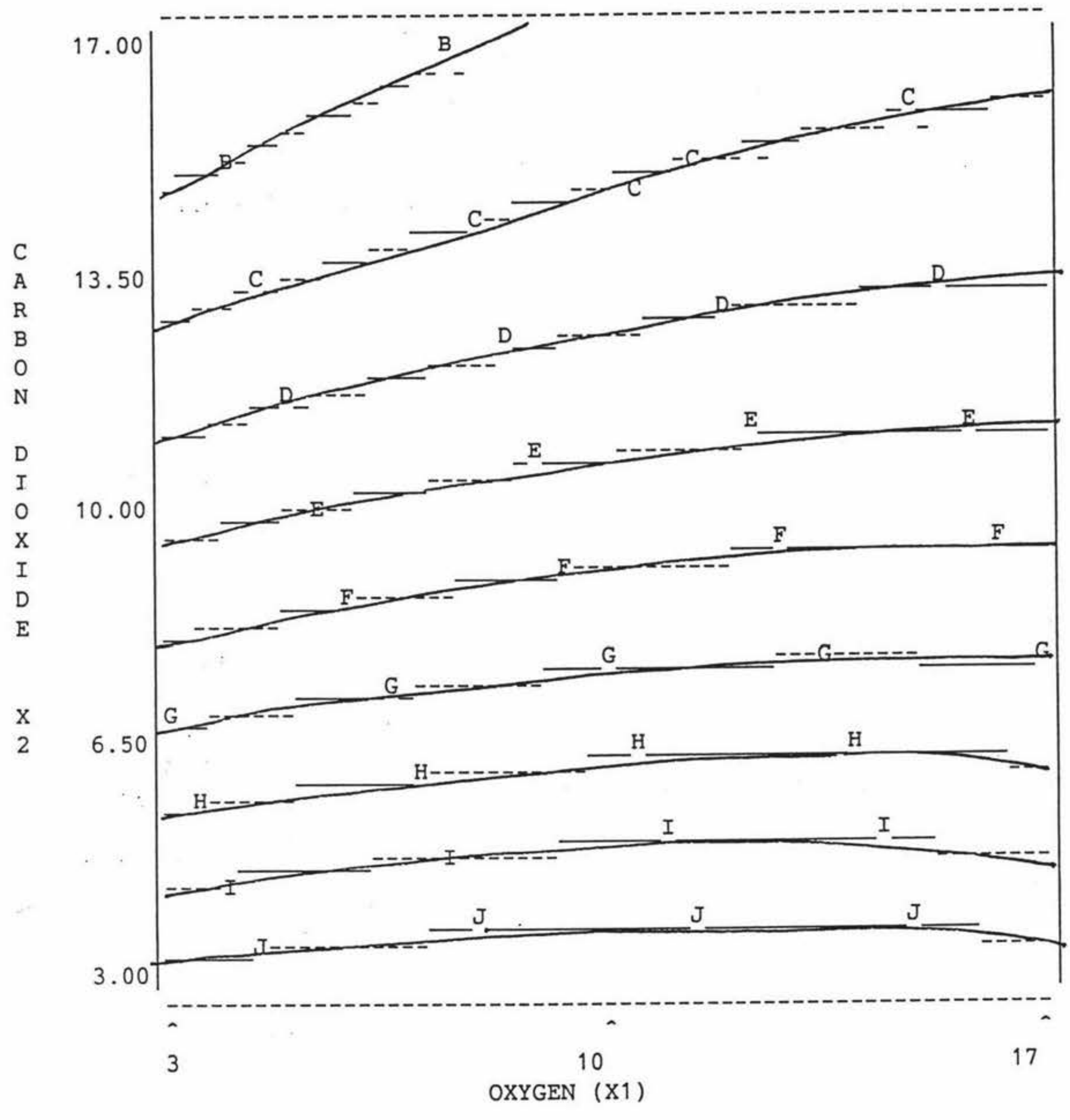
4.2% of the variation is experimental error
7.1% of the variation is unexplained by either the Model or the Experimental error.

Analysis of variance indicates the First Order reaction is significant at greater than 99% confidence.

RSM developed a topographical map of carbon dioxide respiration rates as shown in Figure 5.4. The rate of CO_2 production decreased as the percentage of oxygen present in the atmosphere increased, a similar finding to that reported by Yang & Chinnan (1988). The rate of CO_2 production would appear to be more affected by the increasing CO_2 in the modified atmosphere than by the percentage of oxygen present.

Figure 5.4 RSM Developed Topographical Map of the Carbon Dioxide Respiration Rate at Different Oxygen and Carbon Dioxide Levels for Peeled Garlic.

The X-axis is percent oxygen v/v and the Y-axis is percent carbon dioxide v/v



Where lines on the face of the map are the carbon dioxide respiration rate in moles.kg^{-1}.s^{-1} at the following levels:

A	=	not shown	G	=	1.4×10^{-7}
B	=	2.4×10^{-8}	H	=	1.7×10^{-7}
C	=	4.8×10^{-8}	I	=	1.9×10^{-7}
D	=	7.2×10^{-8}	J	=	2.2×10^{-7}
E	=	9.6×10^{-8}	K	=	not shown
F	=	1.2×10^{-7}	L	=	not shown

The lowest carbon dioxide respiration rates corresponding to the low oxygen respiration rates are presented in Table 5.6, along with the corresponding permeability ratio of the polymer required to maintain the calculated modified atmosphere.

Table 5.6 CO_2 Respiration Rates and Required Permeability Ratio (β) for Different % Carbon Dioxide and % Oxygen Levels.

% Carbon Dioxide	% Oxygen	CO_2 Respiration Rate ($\times 10^{-8}$)	Permeability Ratio β
3.0	3.0	10.5	7.0
5.0	3.0	9.0	4.2
6.5	3.0	7.5	3.2
8.0	3.0	6.0	2.6
10.0	3.0	4.5	2.1

Note: The required polymer permeability ratio (β) was determined by dividing atmospheric oxygen % by the carbon dioxide % of the resulting modified atmosphere (Mannapperuma and Singh (1990)).

The β values above dictate the polymer film to be used and hence the carbon dioxide and oxygen values to be used in the package. The polymers selected were:

	β
Low density polyethylene	4.2[b], 4.3[c]
Polybutylene	2.0[a]

a) Combellick (1985)
b) Mannapperuma and Singh (1990)
c) Pauly (1989)

The bag design (Table 5.8) was calculated using Equations 5.3, 5.4 and 5.5, the oxygen and carbon dioxide permeability coefficients of these polymers (Table 5.7) and the peeled garlic respiration data.

able 5.7 Data Used to Design the Bag Dimensions for Peeled Garlic.

Polymer	Permeability Coefficients		Permeability Ratio
	Carbon Dioxide ($m^2.s^{-1}$)	Oxygen ($m^2.s^{-1}$)	
Low Density Polyethylene[b]	10.45×10^{-12}	2.39×10^{-12}	4.3
Polybutylene[a]	3.8×10^{-12}	1.94×10^{-12}	2.0

) Combellick,(1985)
) Mannapperuma and Singh (1990)

he bag and weights were calculated using a spreadsheet (see Appendix 4 for a etailed printout) and the chosen parameters are set out in Table 5.8.

able 5.8 Calculated Bag Dimensions, Weight of Garlic and Gas Composition for Peeled Garlic.

Polymer	Bag Dimensions	Garlic Weight	CO_2 % V/V	O_2 % V/V
Polybutylene	100mmx200mmx70μ	400g	9	3
LDPE	200mmx200mmx70μ	670g	5	3

he two types of package were made up and used in the storage trials.

.3.3 <u>Storage Trial Evaluation</u>

he results of the weekly inspection of the packages of garlic at each storage ımperature are presented in Table 5.9. Mould was not detected; however, the garlic loves turned translucent and were visually unacceptable after 14 days at 17°C. The as composition of the packs at this temperature was very high in CO_2 (34%-36% CO_2 nd 1.3%-1.4% O_2). However, since the package design was based on data obtained t 5°C, the storage trial was continued at the lower temperatures.

Table 5.9 Days at the Specified Temperature until the Garlic was Unacceptable.

	Temperature					
	Polybutylene			Polyethylene		
	5°C	10°C	18°C	5°C	10°C	18°C
Shelflife (Days)	>49	42	14	>49	42	14

The packaged garlic from the 5°C storage was evaluated for:

- Total Plate Count, and Mould and Yeast.
- Flavour and texture
- Composition of the gas atmosphere

The results of the microbial analysis are detailed in Tables 5.10 and 5.11

Total Plate Counts of 10^4 to 10^5 and mould counts of 10^2 to 10^3 were found on the garlic.

Table 5.10 Results of the Taste Panel Evaluation of Peeled Garlic Stored at 5°C.

Panellist No.	Garlic Flavour (Compared to Control = 5)			
	Polyethylene		Polybutylene	
	Atm	Flushed	Atm	Flushed
1	4	6	5	5
2	5	5	5.5	6
3	5	5	5	4
4	3	2	4	2
5	4	4	3	3
Average	4.2	4.4	4.5	4.0

The garlic flavour would appear to be comparable to the control (fresh garlic), but the comments on the panel sheets indicated the presence of off odours and "different" flavours. These comments are detailed below;
Polyethylene, atmosphere;

"smell on opening bag - hot taste"
"smells on opening bag - tastes hot"
"bad smell on opening bag"
"very hot, OK if you ignore the smell"
"bitter and not the same smell"

Polyethylene, flushed;
"bitter appears hotter than control"
"slightly bitter, looks good"
"hot, bad"
"extra hot"

Polybutylene, atmosphere;
"stronger taste, good texture"
"strong, sharper then control"
"strong garlic flavour, bag smelt after opening"
"that's more like garlic"
"hot"

Polybutylene, flushed;
"slightly sharp taste, not too bad"
"hot after taste"
"cloves a little dry"
"very hot but slight garlic flavour"
"a milder garlic flavour"

Table 5.11 Gas Composition of the Garlic Packages after 7 Weeks at 5°C.

Package	CO_2 % V/V	O_2 % V/V
Polyethylene		
Flushed	28.51	1.04
Atmosphere	31.54	1.11
Polybutylene		
Flushed	44.30	1.49
Atmosphere	44.30	1.49

Thus the bag design was not optimal as there was a build-up of CO_2 to high levels, which resulted in the unfavourable comments on flavour and odour by the taste panellists. These flavour and odour changes would be the result of anaerobic respiration occurring at the high CO_2 levels present in the packages. Similar flavour and odour develop have been noted by other researchers (Saltveit (1989), Yang & Chinnan (1988), Kader et al. (1989)). The oxygen concentration in the packages are very much closer to the recommended modified atmosphere level.

The major reason for the failure of the model to correctly predict the package design appeared to be the respiration data rate generated by the RSM experiment, especially the CO_2 respiration rates. This be will further discussed further after the results obtained for sliced onions have been presented.

5.4 Follow-Up Experiment

After the failure to design the package for the peeled garlic using the respiration data, an alternative approach was used. The respirations rates for whole garlic were compared to those of celery from published data (see Table 5.12).

Table 5.12 Comparison of Carbon Dioxide Respiration Rates of Whole Garlic and Celery[a]

Vegetable	CO_2 Respiration Rates (moles.kg^{-1}.s^{-1})(x10^{-8})		
	0°C	5°C	10°C
Celery	3.16	5.68	15.2
Garlic	2.53	5.68	6.30

Note: a) from Hardenburg et al. (1990).

Garlic and celery have very similar CO_2 respiration rates at 0°C and 5°C and a recommended modified atmosphere of 3-4% CO_2 and 5-6% O_2 (Cessari & Tonini, 1984) for garlic is very similar to that for celery (Saltveit (1989)). Thus the package design method used for celery (described in Chapter 4) could be applied to the packaging of the peeled garlic. The package for the peeled garlic was redesigned using information from the celery trials and from the garlic trials described above. A LDPE bag with a similar surface area (800mm) as used for the celery trials and a film thickness of 30μm. The resulting bag size was 200mm x 200mm x 30μ made from LDPE and with 300g of peeled garlic. The packages were then stored at 5°C and the atmosphere composition monitored. Samples were subjected to sensory evaluation at four and six weeks.

The gas composition of the packages is set out in Table 5.13 and after six weeks of the trial the atmosphere was being maintained within the recommended range. The sensory evaluation data obtained from the expect garlic tasters is set out in Table 5.14.

Table 5.13 Gas Composition of the Modified Atmosphere in the 30μ Polyethylene Package During Storage at 5°C.

Time (Days)	Gas Composition			
	CO_2 % V/V		O_2 % V/V	
	Bag no. 1	2	1	2
0	0.03	21.0	0.03	21.0
14	4.2	5.3	2.5	2.1
28	4.4	4.7	2.0	2.6
35	4.7	5.9	3.4	1.7
42	6.2	5.2	2.5	3.5

Table 5.14 Results of the Sensory Evaluation of Peeled Garlic in the Redesigned Bag Stored at 5°C.

Time (Days)	Average Garlic Flavour Scores
0 (control)	5
28	4.0
42	2.7

The garlic flavour at four weeks was similar to the control garlic, but by week six off flavours were being detected similar to those found in the first garlic package storage trials. Some of these comments are detailed below:

"No taste at first"
"Tasted mouldy"
"When first tasted like cabbage, then hot and a bitter taste on the tongue"
"Slight chemical taste then better"
"Slight chemical taste then better, after-taste good"

These comments indicate some anaerobic respiration is occurring, and thus the shelf-life of the designed bag is about 4 weeks only.

5.5 Other Comments

1. The experimental design for the RSM programme (Table 5.2) specified CO_2 and O_2 percentages that could not be obtained from the gas mixer (compare the required levels in Table 5.2 with the initial percentages actually used in Table 5.3). The analysis of the RSM data indicated, however, that the data was sufficiently accurate to give a good model. Using another computer

programme, "Surfer", Topographical and 3-Dimensional diagrams of the actual % CO_2, %O_2 and respiration data were developed. These diagrams can be seen in Appendix 3 and are very similar to the RSM topographical map.

2. A package was designed that was able to maintain the recommended modified atmosphere for the peeled garlic using the method detailed in Chapter 4, while the bag designed by the more sophisticated method used at the beginning of this Chapter did not. The recommended modified atmosphere for garlic (Cessari & Tonini (1966)) would, from the results of the trials described in Section 5.4, be very similar to that required for the peeled garlic. This is not an unexpected result as the difference between unpeeled and peeled garlic is very minimal. The bag design method used in Chapter 4 is still dependent on the availability of a recommended modified atmosphere in the literature, while the method detailed in this chapter should allow the development of the recommended modified atmosphere.

CHAPTER 6

MODIFIED ATMOSPHERE STORAGE OF PARTIALLY PROCESSED ONIONS

1 Dynamic System Determination of Respiration Rates

ɔ investigate the dynamic model of a package set out in Mannapperuma and Singh 991) for determining the respiration rate of sliced and whole peeled onions.

.1.2 Method

eeled Pukekohe Long Keeper onions were obtained from a commercial operator. he onions were peeled using equipment designed to slice the outer dry layers and ıen using compressed air to blast these off the onion. These onions were prepared n commercial premises as follows:

i) Sliced to give 5mm thick slices using a modified ham slicer.

ii) Whole peeled onions

he onions were then packaged in 100 g lots into low density polyethylene bags, 200mm x 150mm x 30μm. The bags of onions were sealed and stored in a chiller set ıt 2°C, with an effective temperature of 1°C to 4°C. During the storage of the onions, ɔags were selected at intervals set out below and subjected to taste panel, nicrobiological and packaging headspace gas analysis, at the following times:

0 days
2
4
7
10
14
18

The whole onions were inspected weekly beyond this period until mould growth was detected.

The packaging headspace gas composition was then used to calculate the respiration rates of the sliced and whole onions using the dynamic package model of Mannapperuma and Singh (1991). This mathematical statement of the dynamic model consists of three equations describing the rate of change of the concentrations of the three gases, oxygen, carbon dioxide and nitrogen inside the polymeric package.

For oxygen:

$$V\frac{dx_1}{dt} = \frac{P_1 A}{b}(c_1 - x_1) - WR_1 \tag{6.1}$$

For carbon dioxide:

$$V\frac{dx_2}{dt} = \frac{P_2A}{b}(c_2-x_2)+WR_2 \tag{6.2}$$

For nitrogen:

$$V\frac{dx_3}{dt} = \frac{P_3A}{b}(c_3-x_3) \tag{6.3}$$

Where

A	=	area of film (m^2)
R	=	respiration rate (moles/kg.s)
P	=	permeability coefficient (m^2/s)
W	=	weight of produce in the package (kg)
c	=	gas concentrations in ambient atmosphere (moles/m^3)
x	=	gas concentrations in package atmosphere (moles/m^3)
b	=	thickness of the film (m)
dx	=	change in gas concentrations in container atmosphere (moles/m^3)
dt	=	time interval (seconds)
V	=	free volume in the container (m^3)

suffixes 1, 2 & 3 denote oxygen, carbon dioxide and nitrogen gases respectively

In addition, the ideal gas equation relation has to be obeyed by the gases inside the package. This provides a restriction on the total pressure of the package such that:

$$p = (x_1+x_2+x_3)RT \tag{6.4}$$

Where

p	=	pressure (Pa)
x	=	gas concentrations in package atmosphere (moles/m^3)
T	=	temperature (K)
R	=	universal gas constant (J/mole.K)

suffixes 1, 2 & 3 denote oxygen, carbon dioxide and nitrogen gases respectively

By reworking equations 6.1 and 6.2 to produce equations 6.5 and 6.6, the respiration rates of the onions can be calculated, knowing the changes in the composition of gases inside the package, as follows:

For oxygen:

$$R_1 = \frac{P_1 A}{Wb}(c_1 - x_1) - \frac{V}{W}\frac{dx_1}{dt} \tag{6.5}$$

For carbon dioxide:

$$R_2 = \frac{V}{W}\frac{dx_2}{dt} - \frac{P_2 A}{Wb}(c_2 - x_2) \tag{6.6}$$

During the initial research undertaken on the packaging of onions, the free volume inside the packages was not measured. The free volume was subsequently measured on the same size bags containing sliced onions using the water displacement method.

6.1.3 Results

6.1.3.1 Package Atmosphere

The oxygen and carbon dioxide levels measured in the bags of sliced onion are set out in Tables 6.1 and 6.3, and for the whole onions in Tables 6.2 and 6.4.

Table 6.1 Oxygen Level inside the polyethylene bag containing 100g of 5mm sliced Pukekohe Longer Keeper onions.

Day	Oxygen Percentage in Packs (% V/V) Bag Number					
	4	1	3	7	16	17
0	20.9	20.9	20.9	20.9	20.9	20.9
2	18.0	17.8	17.8	17.4	17.4	16.2
4	16.2	14.8	16.2	16.5	15.7	13.4
7	11.2	11.2	11.8	14.6	12.7	10.3
10	3.7	7.4	3.5	4.7	8.3	5.1
14	3.0	2.1	2.6	2.7	5.0	3.7
18	2.6	2.1	2.1	3.8	n/a	3.9
24	2.2	2.2	2.2	n/a	n/a	2.3

n/a no results

Table 6.2 Oxygen levels inside the polyethylene bags containing 100g of whole PLK onions.

Day	Oxygen Percentage in Packs (% V/V) Bag Number					
	4	1	3	7	16	17
0	20.9	20.9	20.9	20.9	20.9	20.9
2	13.5	14.5	13.4	12.8	11.0	14.6
4	12.7	13.5	13.2	12.3	10.7	14.1
7	12.9	12.0	13.2	10.7	8.4	12.8
10	12.2	12.1	13.6	10.1	7.7	12.1
14	12.7	11.5	14.6	9.1	5.6	14.8
24	13.8	8.12	9.8	4.9	4.8	7.6

The carbon dioxide levels measured in the bags are detailed in Tables 6.3 & 6.4 for the Pukekohe Long Keeper onions.

Table 6.3 Carbon dioxide levels measured in the polyethylene bag containing 5mm sliced PLK onions.

Day	Oxygen Percentage in Packs (% V/V) Bag Number					
	4	1	3	7	16	17
0	.03	.03	.03	.03	.03	.03
2	1.96	2.41	2.12	2.20	2.31	2.54
4	2.97	3.41	2.76	2.66	2.95	3.05
7	4.91	5.44	4.77	4.13	4.58	4.05
10	5.76	5.94	5.97	6.37	5.87	4.74
14	5.48	6.32	5.88	6.04	6.31	5.08
18	5.32	5.74	5.68	5.75	6.46	5.05
24	6.96	7.80	7.61	8.02	8.55	7.10

Table 6.4 Carbon dioxide levels measured in a polyethylene bag containing 100g of whole onion.

Day	Oxygen Percentage in Packs (% V/V) Bag Number					
	4	10	11	19	21	22
0	.03	.03	.03	.03	.03	.03
2	3.87	3.64	3.64	4.13	4.95	3.61
4	3.47	3.21	3.25	3.62	4.02	3.04
7	3.45	3.39	2.93	4.01	4.35	3.13
10	2.96	2.71	2.52	3.5	4.18	2.81
14	3.41	3.16	2.59	4.21	5.5	3.07
24	3.6	4.64	4.38	6.65	7.4	5.04

6.1.3.2 Calculation of Respiration Rates

The respiration rates of the onion products were calculated using the data from Tables 6.1 to 6.4 and equation numbers 6.5 & 6.6. The respiration rate results for 5mm sliced onion and whole onions are set out in Tables 6.5 to 6.8. The respiration was calculated for each of the time periods measured. A copy of the Quattro Pro spreadsheet used for this calculations can be seen in Appendix 6.1.

Table 6.5 Calculated Oxygen respiration rates for 5mm sliced PLK onions.

Time Period (days)	Oxygen Respiration Rate ($\times 10^{-8}$) [$moles.kg^{-1}.s^{-1}$] Bag Number					
	4	1	3	7	16	17
0 - 2	-4.6	-4.9	-4.9	-5.5	-5.5	-7.4
2 - 4	-2.8	-4.7	-2.5	-21.4	-2.6	-4.4
4 - 7	-5.2	-3.7	-4.6	-2.0	-3.1	-3.2
7 - 10	-7.8	-3.9	-8.6	-1.0	-4.6	-5.4
10 - 14	-0.53	-4.1	-0.67	-1.6	-2.6	-1.0
14 - 18	-0.34	n/a	-0.44	0.86	n/a	0.14
18 - 24	-0.18	n/a	0.06	n/a	n/a	-0.82

n/a no results

Table 6.6 Calculated Carbon Dioxide respiration rates for 5mm PLK onions.

Time Period (days)	Oxygen Respiration Rate (x 10^{-8}) [moles.kg^{-1}.s^{-1}] Bag Number					
	4	1	3	7	16	17
0 - 2	-1.6	-1.6	-1.0	-0.72	-1.0	-0.80
2 - 4	-2.0	-2.1	-2.1	-1.5	-1.7	-1.0
4 - 7	-0.88	-0.52	-1.2	-2.8	-1.3	-0.72
7 - 10	0.22	-0.3	0.07	0.26	-0.34	-0.27
10 - 14	0.12	4.98	0.16	0.23	0.02	1.73
14 - 18	-0.85	n/a	-1.0	2.99	n/a	-1.1

n/a no result

Table 6.7 Calculated Oxygen respirations rates for whole peeled PLK onions

Time Period (days)	Oxygen Respiration Rate (x 10^{-8}) [moles.kg^{-1}.s^{-1}] Bag Number					
	4	10	11	19	21	22
0 - 2	-12	-10	-12	-13	-15	-9.9
2 - 4	-1.2	-1.6	-0.31	-0.78	-0.47	-0.78
4 - 7	0.21	-1.6	n/a	-1.7	-2.4	-1.4
7 - 10	-0.73	.010	0.416	-0.62	-0.68	-0.73
10 - 14	0.39	-0.47	0.78	-0.79	-1.7	2.1
14 - 24	0.34	-1.1	-1.5	-1.3	-0.24	-2.2

n/a no result

Table 6.8 Calculated Carbon Dioxide respiration rates for whole peeled PLK onions

Time Period (days)	Oxygen Respiration Rate (x 10^{-8}) [moles.kg^{-1}.s^{-1}] Bag Number					
	4	10	11	19	21	22
0 - 2	5.98	5.63	5.63	6.39	7.67	5.58
2 - 4	-0.62	-0.67	-0.60	-0.79	-1.4	-0.88
4 - 7	-0.02	0.19	-0.33	0.41	0.03	0.01
7 - 10	-0.50	-0.70	-0.42	-0.53	-0.17	-0.33
10 - 14	0.36	0.36	0.06	0.56	1.03	0.21
14 - 24	0.15	1.16	1.4	1.91	1.48	1.54

The respiration rates calculated in Tables 6.5 to 6.8 have a number of unexpected negative values. The calculations have been checked both manually and in the computer spreadsheet, but the answers are still negative, which is a physical impossible. The respiration rates are in the correct range if the values were positive. These results support the comments made by Mannapperuma and Singh (1990), that a solution to the set of equations 6.1 to 6.4 depends on the form of the dependence of R_1 and R_2 on x_1 and x_2. If the relationship is simple then an analytical solution can be obtained, but more complex analytical techniques such as Runge-Kutta or predictor-corrector methods are required if the relationship is complex. It would appear that the relationship in sliced onions is complex and a simple method is required to determine the respiration rates. An investigation of the literature provided a carbon dioxide respiration (production) rate of 2.6 x 10^{-8} to 3.5 x 10^{-8} moles.kg^{-1}.s^{-1} at 4-5°C (Hardenburg et al. (1990)) for another variety of onion.

Throughout the shelf life storage trials conducted to obtain the above information, microbiological testing was undertaken. A review of the microbial results (Table 6.9) show very high bacteria, yeast and mould counts, which would add another error to any calculated respiration rates since micro-organisms could use or produce O_2 and/or CO_2. The presence of high microbial numbers after 4 days storage demonstrate the importance of pre-packaging hygiene.

Table 6.9 Microbial results of the 5mm sliced PLK onions throughout the storage period.

Time (day)	Aerobic TPC[a]		Yeast	Mould	Coliform Total[a]
	22°C (log no.)	30°C (No. of Colonies)			
0	5.0		2480	2840	<20
2	7.3		>4000	1440	
4	5.70	6.00	ov/gr	>2000	>4000
7	8.53	8.55	>4000	1020	>4000
10	7.16	7.04	620	1000	
14	8.11	8.03	<20	<20	

a Aerobic Total Plate Counts are Log Numbers
b Yeast, Mould and Coliform are total viable colonies.

6.2 Closed System Respiration Rate Determination

6.2.1 Method

The respiration rate of whole and 5mm sliced onion was determined using the closed system method outlined in Chapter 5, using Response Surface Methodology. The RSM programme was used with 3 variables at 5 levels as summarised in Table 6.10.

Table 6.10 The Variables and Levels for the RSM for Sliced Onions.

Variable	Levels				
Oxygen - % V/V	2.00	5.00	10.00	15.00	18.00
Carbon Dioxide - % V/V	2.00	5.00	10.00	15.00	18.00
Slice Thickness - mm	2.00	5.00	10.00	15.00	18.00

The experiment plan designed by the RSM programme is detailed in Table 6.11.

able 6.11 The Experiment Plan Design by the RSM Programme for Sliced Onions.

Seq. No.	Plan No.	Oxygen % V/V	Carbon Dioxide % V/V	Slice Thickness mm
1	12	10.00	18.00	10.00
2	4	15.00	15.00	5.00
3	6	15.00	5.00	15.00
4	13	10.00	10.00	2.00
5	7	5.00	15.00	15.00
6	10	18.00	10.00	10.00
7	1	5.00	5.00	5.00
8	17	10.00	10.00	10.00
9	11	10.00	2.00	10.00
10	20	10.00	10.00	10.00
11	19	10.00	10.00	10.00
12	3	5.00	15.00	5.00
13	8	15.00	15.00	15.00
14	16	10.00	10.00	10.00
15	9	2.00	10.00	10.00
16	5	5.00	5.00	15.00
17	14	10.00	10.00	18.00
18	15	10.00	10.00	10.00
19	2	15.00	5.00	5.00
20	18	10.00	10.00	10.00

The Ajee jars with the onions in were kept at 4-5°C and the change in carbon dioxide and oxygen levels determined over several days storage using the gas chromatograph. The respiration rates at any instant were calculated by mass balance of the gas components using the following equations.

For oxygen:

$$R_1 = \frac{V}{W}\frac{dx_1}{dt} \tag{6.7}$$

For carbon dioxide:

$$R_2 = \frac{V}{W}\frac{dt_2}{dt} \tag{6.8}$$

6.2.2 Results

The changes in the gas composition measured are detailed in Table 6.12. Several of the experimental runs had to be repeated due to the jar/lid seal leaking. Considerable difficulty was also encountered at high CO_2 levels with an apparent decrease in the CO_2 both in the initial trial and subsequent repeats. An investigation into the reason for the decrease in the CO_2 levels indicated that CO_2 will be absorbed into any moisture that may be present on the surface of the sliced onion. At 5°C and with the higher levels of CO_2 used in this experiment, up to 25% of the CO_2 may be absorbed into the moisture of the cut surface (Jones (1989)). The trials outlined in Table 6.11 were duplicated, although this was not strictly necessary as the RSM has built-in error measurement analysis by using a number of runs with the same level for the variables (Run Nos. 8, 10, 11, 14, 18, & 20).

The weight of onions used and the free volume were recorded (Table 6.13), and the CO_2 and O_2 respiration rates calculated using Quattro Pro spreadsheet similar to that in Appendix 1 (Table 6.14). The data was analyzed by the RSM programme and the confidence statement from the programme for the carbon dioxide and oxygen respiration rate model developed are set out in Table 6.15.

Table 6.12 Time and Gas Composition Data from the Response Surface Methodology Experiment on Sliced Onion Stored at 5°C.

Run No. (Jar No.)	Time In (hours)	Time Out (hours)	Carbon Dioxide		Oxygen	
			Initial % V/V	Final % V/V	Initial % V/V	Final % V/V
1A	11.86	86.06	13.9	13.9	15.5	11.5
1B	11.94	86.14	13.1	12.3	15.3	12.3
2A	leaked					
2B	17.26	105.93	10.5	14.3	10.1	3.00
3A	12.33	86.40	8.9	9.9	4.9	1.0
3B	12.40	86.51	8.9	9.9	5.2	2.1
4A	13.96	88.43	10.9	12.2	9.7	5.3
4B	14.03	88.51	10.9	11.7	9.4	6.1
5A	leaked					
5B	15.83	88.68	11.1	12.5	10.1	4.6
6A	12.98	86.85	1.8	4.8	11.6	7.6
6B	13.55	86.93	1.6	4.2	12.0	6.5
7A	19.86	92.21	4.5	6.7	5.9	1.0
7B	19.77	92.29	4.3	6.2	7.2	3.0
8A	20.32	92.59	9.2	9.7	18.1	15.1
8B	20.25	92.40	9.8	11.4	18.1	12.6
9A	19.96	92.12	4.0	7.8	5.1	0.5
9B	20.05	91.98	3.7	6.0	6.5	2.3
10A	16.48	81.89	13.6	14.5	6.8	4.7
10B	16.62	81.96	14.5	15.7	6.7	3.0
11A	16.35	88.76	10.1	10.3	11.0	8.7
11B	16.83	106.80	10.0	14.3	8.6	3.7
12A	20.48	92.70	17.6	15.5	10.7	7.7
12B	20.57	92.79	16.3	15.1	11.3	8.1
13A	13.44	87.02	9.3	10.3	10.8	7.3
13B	13.62	88.16	7.1	9.2	12.0	8.9
14A	20.16	110.18	14.3	16.4	6.1	1.3
14B	20.25	110.26	13.0	17.1	6.1	0.8
15A	leaked					
15B	18.16	107.28	13.8	13.9	15.3	11.7
16A	20.80	111.92	4.0	6.4	15.9	12.8
16B	20.88	112.24	4.0	7.0	16.2	11.4
17A	17.41	107.31	10.6	13.4	10.6	4.1
17B	17.56	107.43	10.2	14.4	10.2	3.1
18A	leaked					
18B	21.06	93.05	4.1	6.6	15.6	10.8
19A	17.56	107.80	10.2	14.0	10.2	3.6
19B	17.64	107.89	10.0	12.8	10.4	3.0
20A	17.93	107.61	10.1	11.9	10.2	7.5
20B	17.85	107.72	10.9	14.1	9.6	3.1

able 6.12 Time and Gas Composition Data from the Response Surface Methodology Experiment on Sliced Onion Stored at 5°C.(Continued)

Run No. (Jar No.)	Time In (hours)	Time Out (hours)	Carbon Dioxide		Oxygen	
			Initial % V/V	Final % V/V	Initial % V/V	Final % V/V
REPEAT RUNS						
1A	16.15	59.01	15.3	13.4	15.0	14.0
	16.15	86.66	15.3	14.0	15.0	13.2
1B	16.23	59.12	16.4	13.5	14.7	14.4
	16.23	86.93	16.4	14.3	14.7	13.5
3A	16.47	59.22	10.7	9.1	2.5	2.5
	16.47	87.02	10.7	9.4	2.5	1.4
3B	16.57	59.33	10.4	9.9	2.1	1.7
	16.57	87.11	10.4	11.2	2.1	1.3

Table 6.13 Weight and Free Volume Data for the RSM Experiment.

Jar No.	Weight (grams)	Free Volume in Jar (mL)
1A	152.0	876
1B	151.1	879
2A		
2B	152.7	870
3A	151.9	874
3B	154.7	874
4A	152.6	887
4B	152.7	873
5A		
5B	153.3	841
6A	151.4	883
6B	151.9	872
7A	144.7	888
7B	152.2	879
8A	151.8	881
8B	154.3	872
9A	146.1	883
9B	151.9	883
10A	151.9	880
10B	156.2	872
11A	146.8	810
11B	152.7	873
12A	158.5	872
12B	157.9	867
13A	151.8	874
13B	150.4	880
14A	154.6	894
14B	148.6	891
15A		
15B	154.41	880
16A	147.0	895
16B	154.4	877
17A	149.8	877
17B	153.6	880
18A		
18B	154.9	874
19A	150.0	877
19B	151.9	896
20A	151.6	877
20B	151.1	875
Repeats		
1A & 1B as above		
3A & 3B as above		
12A	154.0	872
12B	73.0	954

Table 6.14 The Calculated Carbon Dioxide and Oxygen Respiration Rates for Peeled Garlic.

Jar No.	Calculated Respiration Rates	
	Carbon Dioxide (moles.$kg^{-1}.s^{-1}$) (x 10^{-8})	Oxygen (moles.$kg^{-1}.s^{-1}$) (x 10^{-8})
1A	-0.04	3.84
1B	-0.72	2.86
2A		
2B	2.99	5.60
3A	1.02	3.73
3B	2.05	2.83
4A	1.15	4.10
4B	0.67	3.08
5A		
5B	1.29	5.04
6A	2.81	3.85
6B	2.50	5.24
7A	2.23	5.06
7B	1.78	4.06
8A	0.53	2.91
8B	1.47	5.26
9A	3.81	4.64
9B	2.30	4.21
10A	1.00	2.25
10B	1.28	3.76
11A	0.19	2.09
11B	3.32	3.76
12A	-1.90	2.81
12B	-1.10	2.92
13A	0.98	3.36
13B	1.98	2.92
14A	1.64	3.75
14B	1.33	4.30
15A		
15B	0.13	2.80
16A	1.93	2.54
16B	2.27	3.67
17A	2.24	5.18
17B	3.25	5.55
18A		
18B	2.32	4.60
19A	2.96	5.22
19B	2.23	5.93
20A	1.44	2.08
20B	2.51	5.13
Repeats		
1A	1.31	1.85
1B	2.00	2.43
3A	8.56	2.77
3B	3.09	1.06
12A	8.11	1.67
12B	4.34	2.26

Table 6.15 RSM Confidence Statement for the Sliced Onion Respiration Rate Experiment.

Factor	Respiration Model	
	CO_2	O_2
Standard Error	0.834	0.973
Multi. Correlation Coef.	0.820	0.706
% Variation Explained by Model	67.21%	49.798%
% By First Order Effects	54.35%	18.67%
% By Second Order Effects	8.84%	20.73%
% By Interaction Effects	4.02%	10.39%
% By of Variation is Experimental Error	17.99%	19.00%
% Unexplained	14.80%	31.12%

The model developed for the CO_2 respiration rate explained 67% of the variation, with 18% being due to experimental errors and about 15% not being able to be explained. The O_2 respiration rate model was less accurate with only 50% of the variation being explained by the model, 19% being due to experimental error and a large 31% of the variation not being explained by the model. The negative CO_2 respiration numbers (Table 6.14) are impossible if the onions are still living. The RSM programme generated a number of topographical maps based on these two models and these are in Appendix 6 (Figures A6.1 to A6.14). The following comments on these topographical maps are only brief as the accuracy of the maps is the same as the model they are based on.

Figures A6.1 to A6.4 are XY graphs that show the likely effect of changing O_2 levels on the CO_2 and O_2 respiration rates of 5mm, 10mm, and 15mm thick onion slices at a constant 2% or 4% CO_2 level. The general pattern is similar in all four maps with an increase in CO_2 and O_2 respiration rates as the onion slice thickness is reduced from 15mm to 10mm, followed by a decrease in both respiration rates as the slice thickness is further reduced. The increase in respiration rates with some processing was expected and has been noted by other researchers (Watada et al. (1990) and Rolle & Chism (1987)), and the decreasing respiration rates of the thinner onion slices could be due to increasing cellular damage to the onion cells from the slicing operation.

This tendency for the O_2 and CO_2 respiration rates to increase and then decrease is also shown in the topographical maps (Figures A6.5 to A6.14). These maps shown the effect of different CO_2 and O_2 levels on the CO_2 and O_2 respiration rates for the following slice thicknesses: 18mm, 15mm, 10mm, 5mm, 2mm. All the maps show a similar trend with the respiration rates increasing with decreasing oxygen levels, especially at higher (above 10%) CO_2 levels. At high O_2 and high CO_2 levels the respiration rates are at their lowest.

6.2.3 Discussion

Some reasons for these poor results could be:

a) The respiration rate measurement was calculated from a closed system with the required atmosphere present at the start of the time period (initial gas concentrations in Table 6.11), but with a very different gas composition by the end of the time period (compare the initial and final gas compositions in Table 6.12). A more accurate method of measuring the respiration rates is required, possibly the open system used by Mannapperuma and Singh (1990), with a constant flow of gas of the required composition passing over the product with the change in the gas composition being measured. A measurement similar to this system was set up but the gas mixer used would only generate the required gas composition at flow rates too fast to obtain meaningful measurements from the relatively slow respiring vegetables investigated. The set-up did work for high respiring produce like mushrooms but the accuracy was not checked.

b) The gas mixer was unable to accurately and consistently produce the required gas composition for flushing the Ajee jars as can be seen by comparing the desired gas compositions in Table 6.11 with the actual initial gas composition in Table 6.12.

c) The measured respiration rates of the sliced onion may have been confounded with the respiration rates of any bacteria that were present. The onions, after peeling, were given the required wash sequence before slicing but not after. If contamination did occur then growth would have been rapid as can be seen in the earlier onion experiment (Table 6.9).

d) CO_2 gas dissolving into the water and moist cut surface of the sliced onion is a problem. This may be overcome by multiple flushing of the individual containers.

e) No information is available on the effects of the damage to the onion cell structure of the slicing operation especially with the thinner slices, although the results do indicate that the thinner slices had decreased respiration rates (see Figures A6.1 to A6.4).

The full printout from the RSM programme can be seen in Appendix 6.2, along with the following Topographical Maps and the XY plots discussed above:

a) O_2 respiration rates & CO_2 respiration rates on O_2 & CO_2 axis with slice thickness held constant at 2mm, 5mm, 10mm, 15mm, & 18mm.

b) XY plots of changes in O_2 respiration with slice thickness and changing O_2 concentrations, at CO_2 concentrations of 2% and 4%.

c) XY plots of changes in CO_2 respiration with slice thickness and changing O_2 concentrations, at CO_2 concentrations of 2% and 4%.

The regions on the topographical maps to investigate would appear to be where the RQ = 1 and the respiration rates are minimal. For example, in the topographical map for the slice thickness of 5mm, the regions bounded by E & F in the CO_2 respiration map and D & E in the O_2 respiration map. These give the regions of 15-16% CO_2 & 2-4% O_2, and 15-16% CO_2 & 12-14% O_2 respectively as possible modified atmospheres for 5mm sliced onion. These suggested regions have not been evaluated due to the errors in the models. This experiment requires to be repeated using multiple sparging of the Ajee jar with the required atmosphere.

6.3 Summary

6.3.1 Dynamic System Determination of Respiration Rates

The rearrangement of the dynamic model of the package to measure the respiration rates should allow the determination of these rates under the actual physical conditions encountered by the package. The basic data were obtained from a large storage trial and used to calculate the respiration rates. Many of these rates had negative numbers which is not possible if the onion was still respiring. These results supported comments by Mannapperuma and Singh (1990), that a solution to the dynamic model equations (6.1 to 6.4) depends on the form of the dependence of CO_2 and O_2 respiration on the gas composition of the package atmosphere. If the relationship is simple (as was assumed in this study) then an analytical solution can be obtained. However, where the relationship is complex, as is it would appear to be for sliced onions, then more complex analytical techniques such as Runge-Kutta or predictor-corrector methods are required.

This research also demonstrated the critical importance of the pre-packaging processing to reduce the microbial contamination. Poor hygiene can result in bacterial numbers higher than 10^6 per gram of sliced onion within four days of storage.

6.3.2 Closed System Determination Of Respiration Rates

The major problem encountered using this method of determining the respiration rates was the CO_2 dissolving onto the wet cut surfaces of the sliced onion. This resulted in the CO_2 levels reducing when high levels of the gas were present in the jars. The interpretation of the results from the Response Surface Methodology experimental design technique used was difficult as the areas being explored are not commonly investigated, i.e. the high CO_2, and high O_2 regions.

6.3.3 General Comments

The main aim of this research was to develop a model to design modified packages for sliced onions that would be able to accommodate different slice thicknesses. As a result of the difficulty of obtaining suitable respiration data, the development of the MAP for sliced onion was not completed. As the respiration data is the limiting factor, then the mathematical model used to design the celery MA package could be applied to design a suitable package for sliced onions. For this to work, assumptions would have to be made as to a suitable modified atmosphere as there are none presented in the literature for sliced onion.

CHAPTER 7

SUMMARY AND CONCLUSIONS

7.1 Pre-Treatments

The pre-treatment wash of cold water followed by a cold water chlorine wash was very effective in reducing the initial bacterial load on the peeled garlic and celery stalks by 3 to 4 log numbers, ie 10^4 - 10^5 down to 10^1 to 10^2. The effectiveness of this wash treatment was not evaluated with the onions after slicing when the cell exudate provides a good medium for bacterial growth.

The anti-browning treatment was less effective with neither treatment tested (SO_2 and a combination of food acids) completely stopping browning of the cut celery surface. The two treatments also changed the flavour of the celery when compared to fresh untreated produce.

7.2 Pack Construction and Materials

7.2.1 Celery MA Pack Design

A suitable modified atmosphere pack was designed using the analysis of the packaging parameters presented by Mannapperuma and Singh (1990) in planning the experimental studies of the celery in a polymeric film. The recommended celery modified atmosphere of 2% - 4% O_2 and 3% - 4% CO_2 was successfully attained, the celery stalks having a packed shelf-life of two weeks, with an acceptable celery taste after 3 weeks, according to the taste panels conducted. The package was 100mm wide x 400mm long x 70µm thick low density polyethylene film, containing 300g of celery stalks and stored at 5°C. A difference was noted in the celery flavour after four weeks at 5°C between produce packed in air and that packed in a flushed atmosphere of 4.5 -5%CO_2 and 3 - 4% O_2. The difference was very small and favoured the produce packed in air. Packaging the celery stalks in air also represented a major capital saving on the part of the commercial grower, as gas-flush packing equipment would not have to be purchased. Therefore this method of packaging was recommended. The required modified atmosphere was generated inside the pack over a 4 - 5 day period by the celery stalks. This pack is currently being commercialized by the grower.

7.2.2 Peeled Garlic MA Pack Design

The use of the experimental design method Response Surface Methodology for determining the optimum modified atmosphere appeared to work well but the designed package failed to attain the desired gas composition. The CO_2 respiration rate data used in the model was incorrect as the resulting packages developed very high CO_2 levels (30% - 40%). RSM and other experimental design techniques are potentially very powerful tools for determining the optimum modified atmosphere, but their application to determining respiration rates requires more research. Also, more accurate measurement methods are required for the calculation of the O_2 and CO_2 respiration rates.

The method for determining package design parameters using the produce respiration data used in this experiment, was very easy to use especially with a computer spreadsheet. The permeability of the polymer films used should have been tested under the conditions that the films are going to be used, and this data used in the model to ensure more accurate results. The variety and proliferation of permeability units and values for gas permeability was a major problem, but the method and units used by Mannapperuma and Singh (1990) have much to recommend them as they are simple and easy to use.

A successful pack was designed by going back to the literature and using a recommended modified atmosphere, which was similar to that for celery, and thus using the pack design parameters determined for the celery stalks. The modified package design was 200 wide x 200mm x 30μm thick low density polyethylene film containing 200g of peeled garlic. Cessari and Tonini, (1966) reported a very good shelf-life (88 days) for whole garlic stored in 5-6% O_2 and 3-4% CO_2 at 2-3 °C. Thus a longer shelf-life may be possible by storing the peeled garlic at this lower temperature. This would require a redesign of the package for the peeled garlic. The peeled garlic package is presently being evaluated on the New Zealand market.

7.2.3 Sliced Onion Package Design

The initial research on onion package design involved using the Mannapperuma and Singh (1990) model for dynamic behaviour of the package to determine the sliced onion respiration rates. This study used data from a previous experiment to design a MA package for sliced onion. The dynamic model, in the form it was used, was not able to determine the respiration rates for the sliced onions as it produced a large number of impossible negative values. This research did, however, highlight the importance of being able to accurately measure the free volume inside the package. The use of a measured amount of methane gas, as recommended by Mannapperuma and Singh (1990), produced variable results, so was not used. The use of a noble gas may prove more accurate and should be evaluated.

The second part of the research to design a MA package for sliced onion used RSM to determine the respiration rates of the sliced onion. This data was then to be used to develop the optimum modified atmosphere for an given slice thickness. The accuracy of the respiration models generated was not very good with only about 50% of the experimental variation being explained by the O_2 respiration rate model, and 67% of the variation by the CO_2 model. Possible reasons for this poor result include:

a) Absorption of CO_2 gas at the higher concentrations into the exudate from the damaged cells of the onion.

b) The gas chromatograph results were not accurate enough.

c) The use of the closed system for measuring the respiration rate with the constantly changing atmosphere not being representative of the actual respiration at the required gas composition for the RSM programme.

d) The presence of bacteria which can grow very rapidly even at the 5°C storage temperatures used in this study.

The interpretation of the RSM topographical maps (Figures A6.5 to A6.14) was difficult, especially as these maps were developed using the models with high percentages of the experimental error and unexplained variation. However, the use of experimental design methodology for exploring the effects of CO_2 and O_2 on the respiration rates of sliced onion is very promising . These methods allow a large region of CO_2 and O_2 values to be explored quickly, while providing information on any interactions that may be occurring.

7.3 Mannapperuma and Singh (1990) Model Evaluation

The basic mathematical model outlined by Mannapperuma and Singh (1990) is very similar to other models developed by other researchers. The application of this model and developments of this model proved very useful in designing MA packages for the celery stalks and the peeled garlic. Unfortunately, a more detailed study of the application of all of the package design methods was not undertaken due to inaccuracy in the respiration rate data. The availability of accurate respiration rate data and the optimum modified atmosphere for minimally processed vegetables are a major block in the more extensive use of the mathematical MA packaging models.

Ethylene was not included in any of the models developed by Mannapperuma and Singh (1990), or in this research, however, it can cause an increase in respiration rates at very low concentrations in minimally processed vegetables. The effect of minimal processing on the effects of ethylene and the subsequent effect on the modified atmosphere are also missing from the models.

7.4 Further Research

Further research should concentrate on the development of simple models to determine the optimum modified atmosphere for minimally processed vegetables and fruit from accurate respiration rate data. The use of experimental design techniques will be important to ensure the effects of CO_2 and O_2 on the respiration rate is fully explored. A recently released computer programme called "Echip" would provide a good starting basis as this computer programme is more sophisticated than the RSM programme and allows far more flexibility in the experimental design. The actual initial CO_2 and O_2 levels can be used in this programme, thus removing one of the sources of experimental error found when using the RSM programme.

The simple packaging design model used to design the celery package should be improved by the application of experimental design techniques. These should remove some of the trial and error experimental work involved in selecting the required bag size. Research needs to continue to establish a data base of the necessary data required to understand and model the behaviour of minimally processed vegetables and fruit, and to determine the optimum pre-packaging procedures, package design and materials in order to maximize product quality, product safety, and acceptable shelf-life (Geeson & Brocklehurst, 1989).

Recent research has highlighted new ceramic-filled polymer films for application to packaging fresh vegetables and fruit (Lee et al., 1992). The properties of these and their application to minimally processed vegetables and fruit need to be explored. Lee et al. (1992) also note that it is important to understand how the ceramic content, ceramic particle size and distribution, the processing conditions and morphology affect the barrier and mechanical properties of these films. The application of these ceramic-filled polymer films to minimally processed vegetables and fruit should be investigated, as it would appear to be possible to tailor these films to provide the required O_2 and CO_2 permeability rates. The ability to select the correct polymer film would allow ready application of MAP mathematical models to designing produce packages.

Further research is required to determine the effects of ethylene on minimally processed vegetables, and the subsequent effects on any modified atmosphere. These effects could be important when determining the respiration rates of these vegetables.

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APPENDIX

APPENDIX 1: "Quattro Pro" spreadsheet of the Calculations of the Respiration Rate of the Peeled Garlic

RESPIRATION RATE CALCULATION - CLOSED SYSTEM
GARLIC RSM RESULTS

	Free Volume		Weight		Hours			TIME		oxygen				Oxygen Respiration					Carbon Dioxide				Carbon Dioxide Respiratio				
	mls	m3	g	kg	start	finish	actual	min	sec	start %	moles/m3	end %	moles/m3	difference	moles/kg.s	average	mg/kg.hr	average	start %	moles/m3	end %	moles/m3	difference	moles/kg.s	average	mg/kg hr	average
(Column N°)	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB
a1	870	0.00087	150.66	0.15066	16.37	38.37	22.01	1320.32	79219.08	14.85	6.50	7.62	3.34	3.17	2.31E-07		26.59		4.94	2.16	7.85	3.44	1.27	9.29E-08		14.71	
b1	870	0.00087	150.66	0.15066	16.46	38.91	22.46	1347.42	80845.20	14.61	6.40	7.50	3.28	3.11	2.22E-07	2.27E-07	25.62	26.10	4.68	2.05	8.43	3.69	1.64	1.17E-07	1.05E-07	18.58	16.65
a2	870	0.00087	150.45	0.15045	17.27	39.62	22.35	1340.93	80456.04	10.46	4.58	5.18	2.27	2.31	1.66E-07		19.14		9.12	3.99	11.59	5.08	1.08	7.77E-08		12.31	
b2	870	0.00087	150.06	0.15006	17.34	39.86	22.52	1350.93	81055.80	10.20	4.47	6.15	2.69	1.77	1.27E-07	1.47E-07	14.61	16.88	8.67	3.80	10.90	4.77	0.98	6.98E-08	7.38E-08	11.06	11.69
a3	870	0.00087	150.71	0.15071	11.37	39.19	27.83	1669.63	100177.9	9.96	4.36	3.91	1.71	2.65	1.53E-07		17.59		16.89	7.40	17.55	7.68	0.29	1.67E-08		2.64	
b3	870	0.00087	150.11	0.15011	11.48	37.28	25.81	1548.38	92903.04	9.29	4.07	2.35	1.03	3.04	1.90E-07	1.71E-07	21.84	19.71	17.77	7.78	18.35	8.04	0.25	1.58E-08	1.62E-08	2.51	2.57
a4	870	0.00087	150.19	0.15019	11.79	37.36	25.57	1534.31	92058.84	14.71	6.44	8.47	3.71	2.73	1.72E-07		19.81		14.48	6.34	14.64	6.41	0.07	4.41E-09		0.70	
b4	870	0.00087	150.01	0.15001	11.86	37.43	25.57	1534.25	92054.88	14.90	6.52	7.40	3.24	3.28	2.07E-07	1.89E-07	23.84	21.82	13.97	6.12	15.83	6.93	0.81	5.13E-08	2.79E-08	8.13	4.41
a5	870	0.00087	150.20	0.1502	17.41	39.98	22.57	1354.01	81240.84	10.97	4.80	4.80	2.10	2.70	1.93E-07		22.19		8.77	3.84	10.90	4.77	0.93	6.65E-08		10.53	
b5	870	0.00087	150.04	0.15004	17.47	40.08	22.60	1356.18	81370.80	10.45	4.58	4.83	2.12	2.46	1.75E-07	1.84E-07	20.20	21.20	8.78	3.84	10.76	4.71	0.87	6.18E-08	6.41E-08	9.79	10.16
a6	870	0.00087	150.25	0.15025	13.10	37.51	24.41	1464.47	87868.08	3.20	1.40	0.08	0.04	1.37	9.00E-08		10.37		9.70	4.25	11.39	4.99	0.74	4.88E-08		7.72	
b6	870	0.00087	150.88	0.15088	13.20	37.59	24.39	1463.60	87815.88	3.64	1.59	1.33	0.58	1.01	6.64E-08	7.82E-08	7.65	9.01	9.20	4.03	9.87	4.32	0.29	1.93E-08	3.40E-08	3.05	5.39
a7	870	0.00087	150.79	0.15079	16.91	37.69	20.79	1247.18	74831.04	5.67	2.48	0.90	0.39	2.09	1.61E-07		18.55		14.73	6.45	15.38	6.73	0.28	2.19E-08		3.48	
b7	870	0.00087	150.06	0.15006	16.98	37.81	20.83	1249.82	74989.08	5.73	2.51	1.34	0.59	1.92	1.49E-07	1.55E-07	17.12	17.84	14.42	6.31	16.10	7.05	0.74	5.69E-08	3.94E-08	9.01	6.24
a8	870	0.00087	150.08	0.15008	13.98	37.84	23.85	1431.18	85870.80	17.27	7.56	9.35	4.09	3.47	2.34E-07		26.97		8.97	3.93	12.00	5.25	1.33	8.96E-08		14.19	
b8	870	0.00087	150.24	0.15024	14.12	37.91	23.79	1427.35	85641.12	17.16	7.51	10.65	4.66	2.85	1.93E-07	2.13E-07	22.20	24.59	8.43	3.69	11.19	4.90	1.21	8.17E-08	8.56E-08	12.94	13.57
a9	870	0.00087	150.71	0.15071	17.55	40.39	22.84	1370.35	82221.12	10.45	4.58	4.58	2.01	2.57	1.80E-07		20.79		8.36	3.66	11.28	4.94	1.28	8.98E-08		14.22	
b9	870	0.00087	150.11	0.15011			0.00	0.00	0.0		0.00		0.00	0.00		1.8E-07	0.00	10.39		0.00		0.00	0.00		8.98E-08		14.22
a10	870	0.00087	150.16	0.15016	16.77	33.34	16.57	994.30	59658.12	9.58	4.19	4.85	2.12	2.07	2.01E-07		23.17		9.66	4.23	10.99	4.81	0.58	5.66E-08		8.96	
b10	870	0.00087	150.81	0.15081	16.86	33.49	16.63	997.63	59857.92	9.70	4.25	4.68	2.05	2.20	2.12E-07	2.07E-07	24.41	23.79	9.22	4.04	10.55	4.62	0.58	5.61E-08	5.63E-08	8.89	8.92
a11*	790	0.00079	150.09	0.15009	14.56	38.00	23.43	1406.00	84359.88	5.86	2.57	4.67	2.04	0.52	3.25E-08		3.75		4.91	2.15	7.83	3.43	1.28	7.98E-08		12.64	
b11	775	0.000775	150.36	0.15036	14.68	38.08	23.40	1404.05	84243.24	5.55	2.43	0.91	0.40	2.03	1.24E-07	7.84E-08	14.32	9.03	4.59	2.01	9.64	4.22	2.21	1.35E-07	1.08E-07	21.43	17.03
a12	870	0.00087	150.42	0.15042	16.62	38.04	21.42	1284.97	77097.96	10.67	4.67	1.48	0.65	4.02	3.02E-07		34.78		2.60	1.14	7.39	3.24	2.10	1.57E-07		24.92	
b12*	870	0.00087	150.47	0.15047	16.74	38.13	21.39	1283.58	77014.80	10.65	4.66	4.43	1.94	2.72	2.04E-07	2.53E-07	23.56	29.17	2.58	1.13	6.58	2.88	1.75	1.31E-07	1.44E-07	20.83	22.88
a13	870	0.00087	150.74	0.15074	16.94	33.57	16.63	997.54	59852.16	9.51	4.16	4.40	1.93	2.24	2.16E-07		24.86		9.84	4.31	11.56	5.06	0.75	7.26E-08		11.50	
b13	870	0.00087	150.20	0.1502	17.01	33.74	16.73	1003.62	60217.20	9.96	4.36	6.07	2.66	1.70	1.64E-07	1.9E-07	18.88	21.87	8.99	3.94	10.27	4.50	0.56	5.39E-08	6.33E-08	8.54	10.02

$C = B \times 10^{-6}$

$E = D \div 1000$

$H = G - F$

$I = H \times 60$

$J = I \times 60$

$L = K \times 0.43759$ (using $PV = nRT$, $n = \%_{gas} \times 0.43759$ at 1 atm & 2°C)

$N = M \times 0.43759$

$O = L - N$

$P = \left(\frac{C}{E}\right) \times \left(\frac{O}{J}\right) \left[R_1 = \frac{V}{W} \times \frac{c_1 - x_1}{t}\right]$

$R = P \times (3600 \times 1000 \times 32)$

$U = T \times 0.43759$

$W = V \times 0.43759$

$X = W - U$

$Y = \left(\frac{C}{E}\right) \times \left(\frac{X}{J}\right) \left[R_2 = \frac{V}{W} \times \frac{x_2 - c_2}{t}\right]$

$AA = Y \times (3600 \times 1000 \times 44)$

APPENDIX 2: Printout from the RSM Programme

	Variables and Levels				
	-1.4	-1.0	0.0	1.0	1.4
X1 = Oxygen	3.00	5.00	10.00	15.00	17.00
X2 = Carbon Dioxide	3.00	5.00	10.00	15.00	17.00

Oxygen and Carbon Dioxide in %v/v

Responses

Y1 = oxygen respiration rate (moles.$kg^{-1}.s^{-1}$)
Y2 = carbon dioxide respiration rate (moles.$kg^{-1}.s^{-1}$)

TAYLOR EXPANSION EQUATION AND CALCULATED BETA COEFFICIENTS USING CODED VALUES FOR X'S

Yi = B0 + B1 *X1 + B2 *X2 + B11*X1^2 + B22*X2^2 + B12*X1*X2

	Y1	Y2
B0 =	18.163	6.945
B1 =	4.688	0.729
B2 =	-0.962	-4.099
B11 =	-2.256	-0.489
B22 =	1.131	0.546
B12 =	-2.865	-0.212

08:49:56 11-06-1991

CALCULATED VERSUS OBSERVED
RESPONSE VALUES FOR YOUR
EXPERIEMENTAL RUNS

	Y1		Y2	
	CA	OB	CA	OB
1	25.554	22.700	12.042	10.500
2	18.163	14.650	5.945	7.375
3	19.034	17.100	2.278	1.620
4	17.899	18.900	3.420	2.790
5	18.163	18.400	6.945	6.410
6	7.177	7.820	4.965	3.400
7	14.253	15.500	2.387	3.940
8	20.304	21.300	7.006	8.560
9	18.163	18.000	6.945	8.980
10	18.163	20.700	6.945	5.630
11	10.447	7.840	10.159	10.800
12	21.728	25.300	13.754	14.400
13	18.163	19.000	6.945	6.330

Y1 - Oxygen Respiration

CONFIDENCE STATEMENTS

Standard Error = 2.802116

Standard Deviation of Replicates = 2.211383
This Equals 12.17 Percent of the Centerpoint Value

Multiple Correlation Coefficient = .9092205

82.65 Percent of the Variation is Explained by the Model

57.2 Percent by First Order (Linear) Effects
15.1 Percent by Second Order (Quadratic) Effects
10.35 Percent by Interaction Effects

6.16 Percent of the Variation is Experimental Error

11.18999 Percent of the Variation is Unexplained
by Either the Model or the Experimental error

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Calculated	F Table (95%)
Regression					
First Order	181.42	2	90.71	18.55	6.64
Second Order	47.91	2	23.95	4.90	6.64
Interaction	32.83	1	32.83	6.71	7.71
Lack of Fit	35.40	3	11.80	2.41	6.59
Exper. Error	19.56	4	4.89		
Total	317.12	12	26.43		

Y2 - Carbon Dioxide Respiration

Standard Error = 1.604957

Standard Deviation of Replicates = 1.296129
This Equals 18.66 Percent of the Centerpoint Value

Multiple Correlation Coefficient = .941821

88.69 Percent by First Order (Linear) Effects
2.58 Percent by Second Order (Quadratic) Effects
.11 Percent by Interaction Effects

4.21 Percent of the Variation is Experimental Error

7.100001 Percent of the Variation is Unexplained
by Either the Model or the Experimental Error

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Calculated	F Table (95%)
Regression					
First Order	137.26	2	68.63	40.85	6.64
Second Order	4.13	2	2.07	1.23	6.64
Interaction	0.18	1	0.18	0.11	7.71
Lack of Fit	11.31	3	3.77	2.24	6.59
Exper. Error	6.72	4	1.68		
Total	159.61	12	13.30		

Figure A2.1 XY Plot of the Effect of Three CO_2 Levels on the Oxygen Respiration Rate at Different Oxygen Levels.

Values for Carbon Dioxide are L = 5%v/v, M = 10%v/v, H = 15%v/v.
The Oxygen respiration Rates are (x 10^{-8}) moles.kg^{-1}.s^{-1}

The graph shows that at higher CO_2 levels and high O_2 levels the O_2 oxygen respiration rate is lower than at lower CO_2 levels. At the low oxygen levels the higher CO_2 levels appear to increase the oxygen respiration rate.

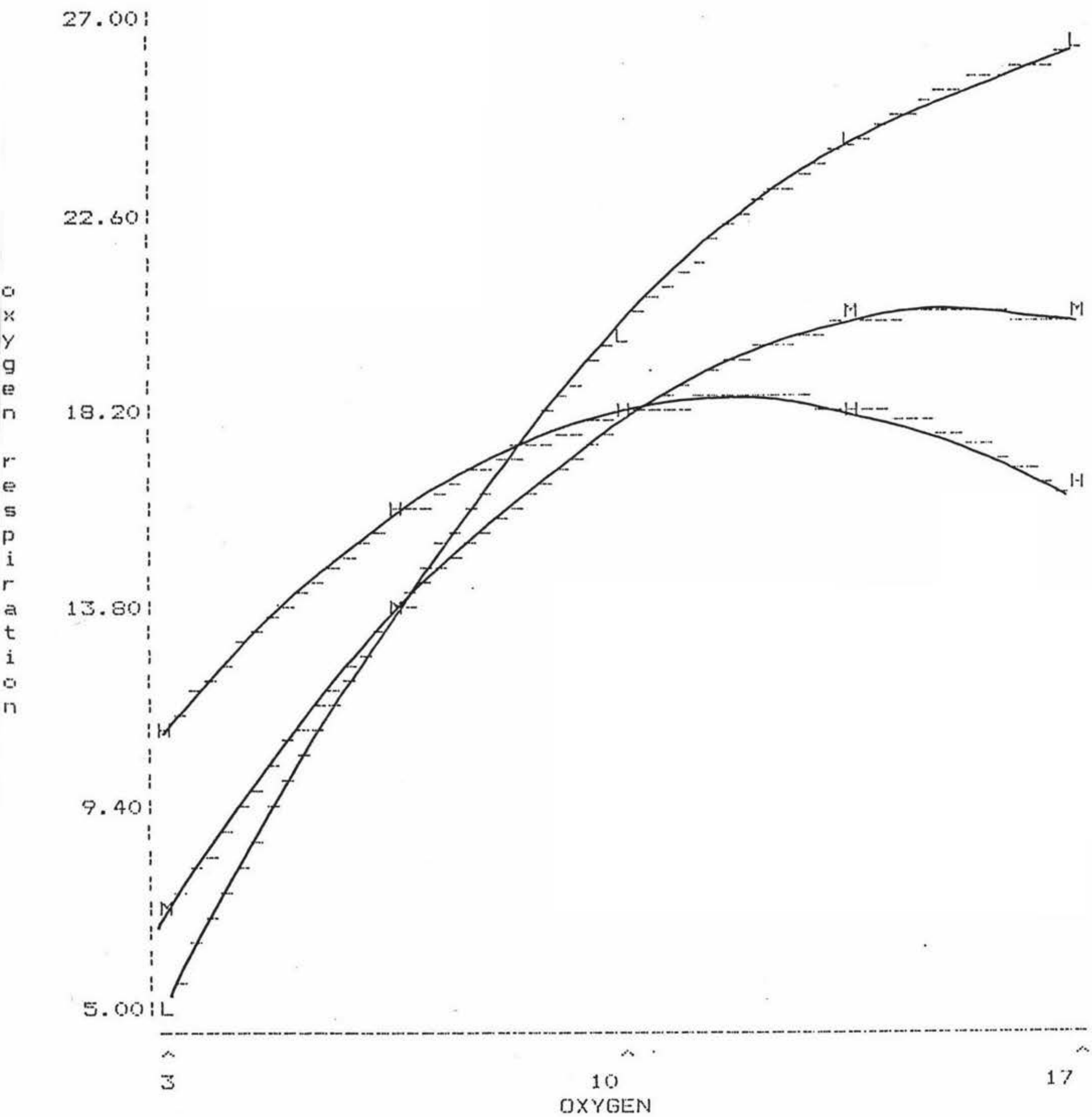


Figure A2.2 XY Plot of the Effect of Three CO_2 Levels on the Carbon Dioxide Respiration Rate at Different Oxygen Levels.

Values for Carbon Dioxide are L = 5%v/v, M = 10%v/v, H = 15%v/v.
The Carbon Dioxide Respiration Rates are (x 10^{-8}) moles.kg^{-1}.s^{-1}

The graph shows that higher CO_2 levels reduce the carbon dioxide respiration rate, whatever the O_2 level.

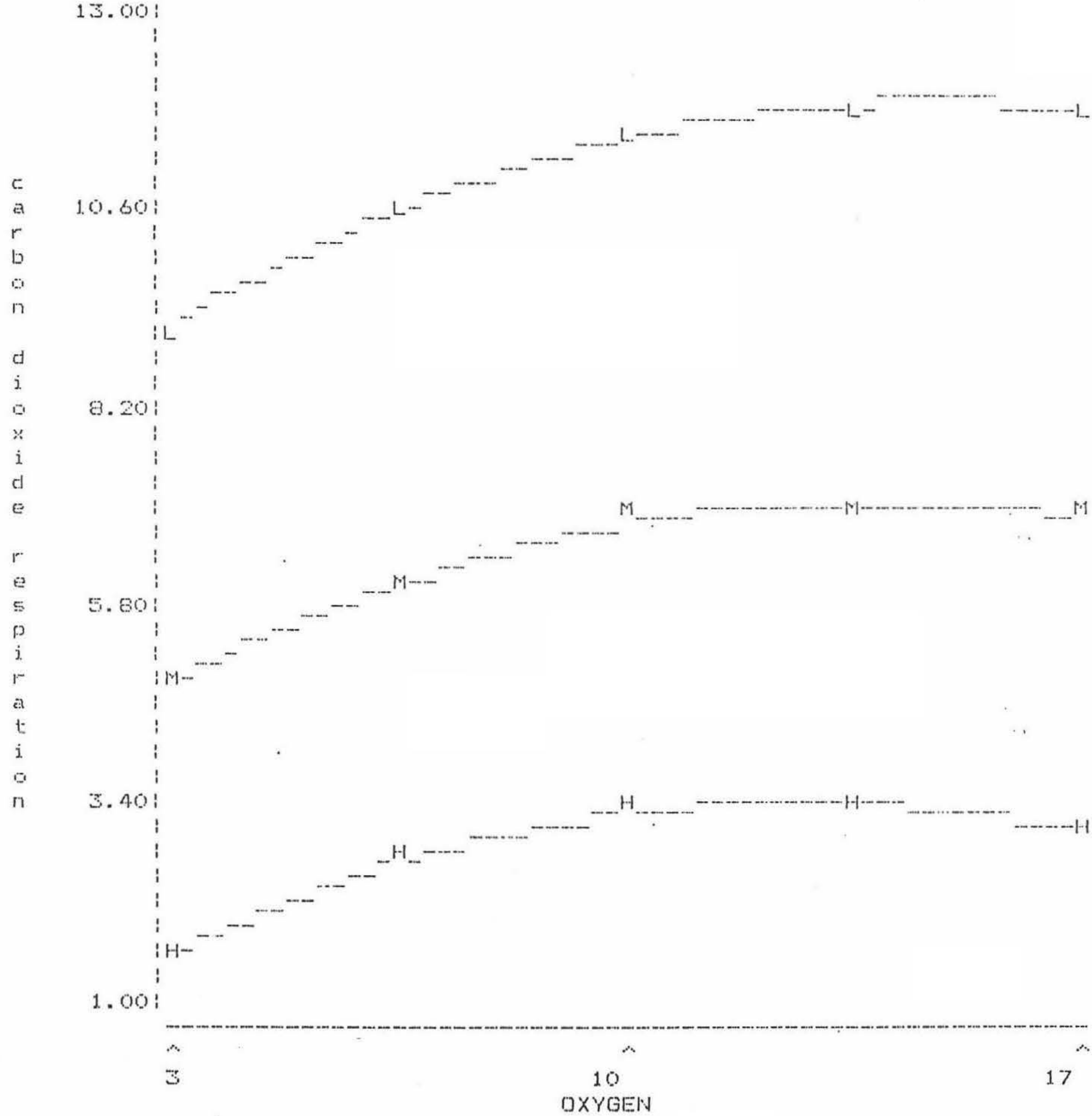


Figure A2.3 XY Plot of the Effect of Three O_2 Levels on the Oxygen Respiration Rate at Different Carbon Dioxide Levels.

Values for Oxygen are L = 5%v/v, M = 10%v/v, H = 15%v/v.
The Oxygen Respiration Rates are (x 10^{-8}) moles.kg^{-1}.s^{-1}

The graph shows that higher CO_2 % v/v in the atmosphere reduce the oxygen respiration rate at 15% and 10% O_2 levels but at a 5% O_2 level the O_2 respiration rate increases.

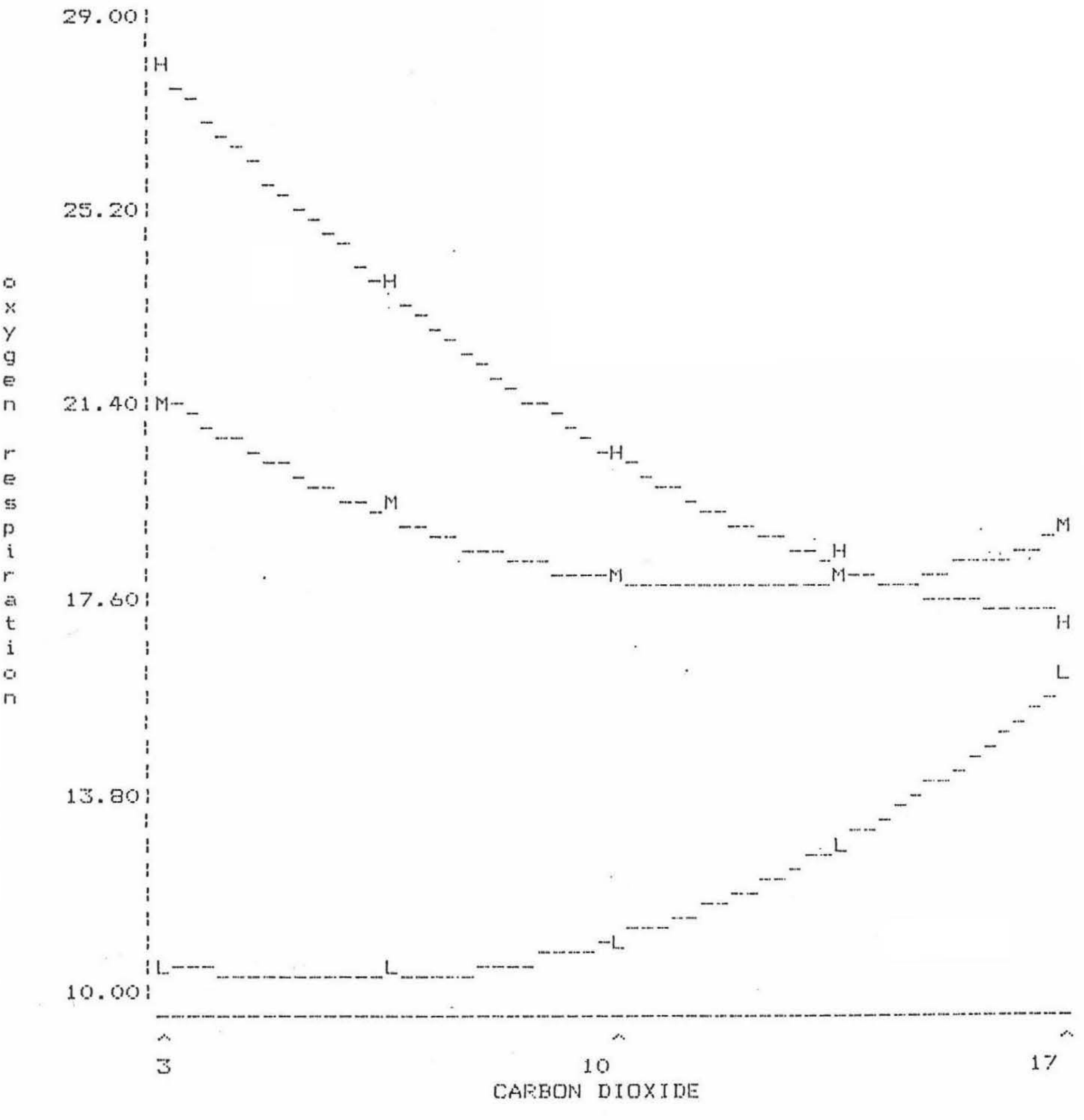
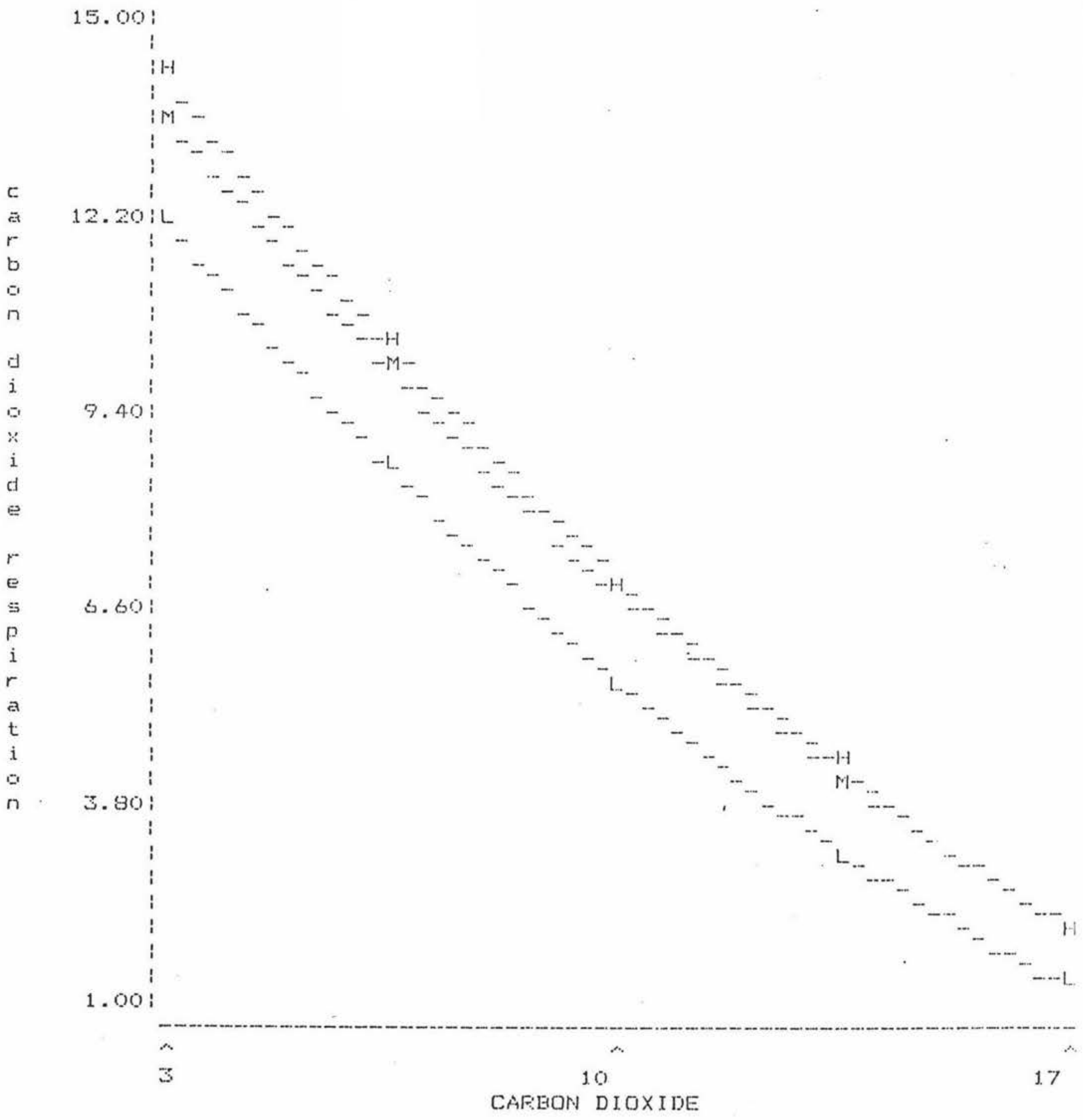


Figure A2.4 XY Plot of the Effect of Three O_2 Levels on the Carbon Dioxide Respiration Rate at Different Carbon Dioxide Levels.

Values for Oxygen are L = 5%v/v, M = 10%v/v, H = 15%v/v.
The Carbon Dioxide Respiration Rates are (x 10^{-8}) moles.kg^{-1}.s^{-1}

The graph shows that higher CO_2 % v/v in the atmosphere reduce the carbon dioxide respiration rate at all three O_2 levels. The level of oxygen appears to have little affect on the CO_2 respiration rate compared to the effect of the increasing CO_2 levels.



APPENDIX 3:

"Surfer" Topographical and 3-Dimensional diagrams developed from the results of the RSM experiment to determine the respiration rates of the peeled garlic. The actual initial O_2 and CO_2 compositions in the jars and the resulting CO_2 and O_2 respiration rate data were used.

Figure A3.1 Oxygen Respiration Rate Topographical Map Drawn by "Surfer"

The Oxygen Repiration Rates (x 10^{-8} moles.kg^{-1}.s^{-1}) are on the face of the map, and the Y-axis is % v/v carbon dioxide and the X-axis being %v/v oxygen.

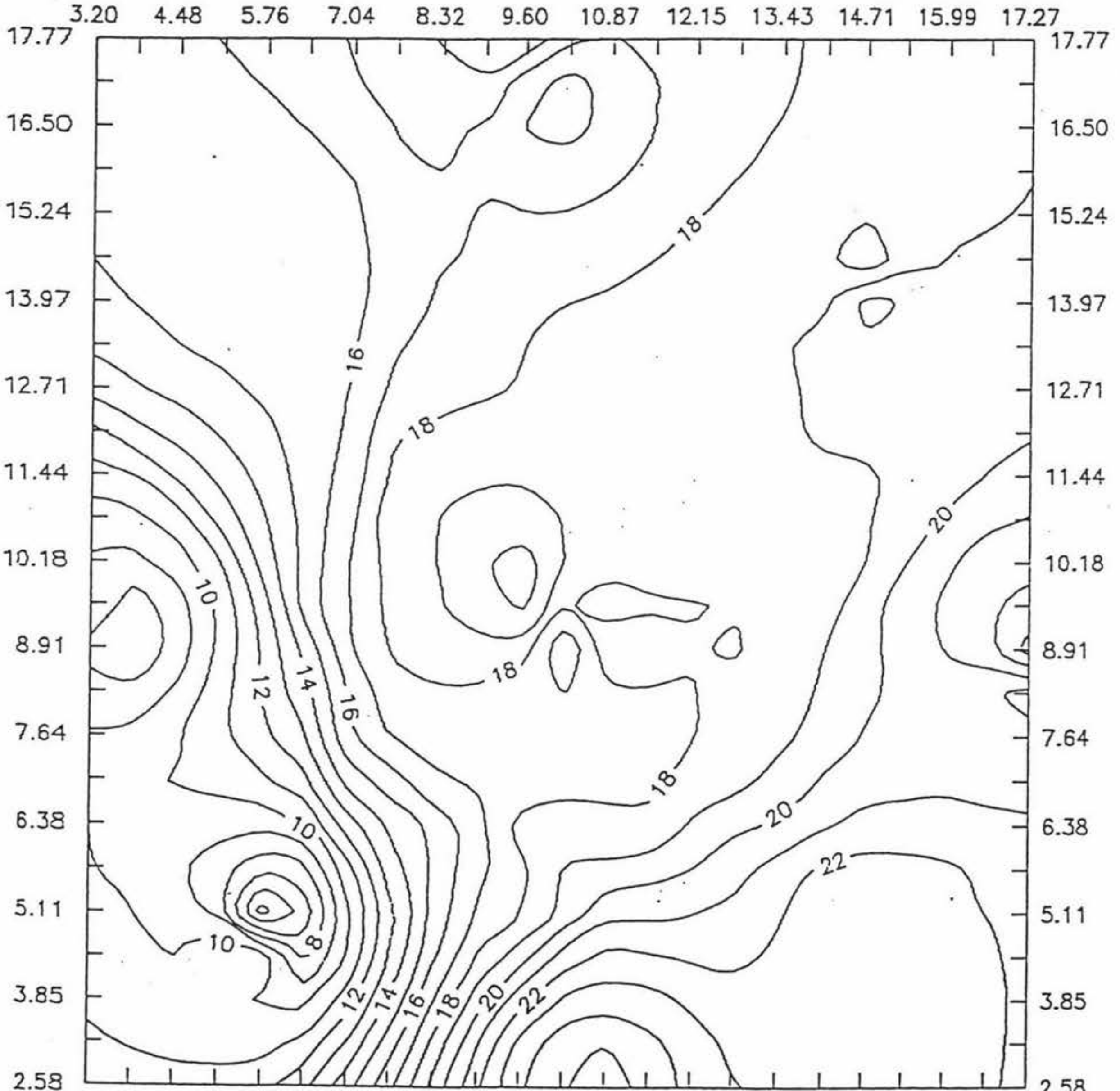


Figure A3.2 Oxygen Respiration Rate 3-Dimensional Diagram Drawn by "Surfer"
(Oxygen Repiration Rates (x 10^{-8} moles.kg^{-1}.s^{-1}))

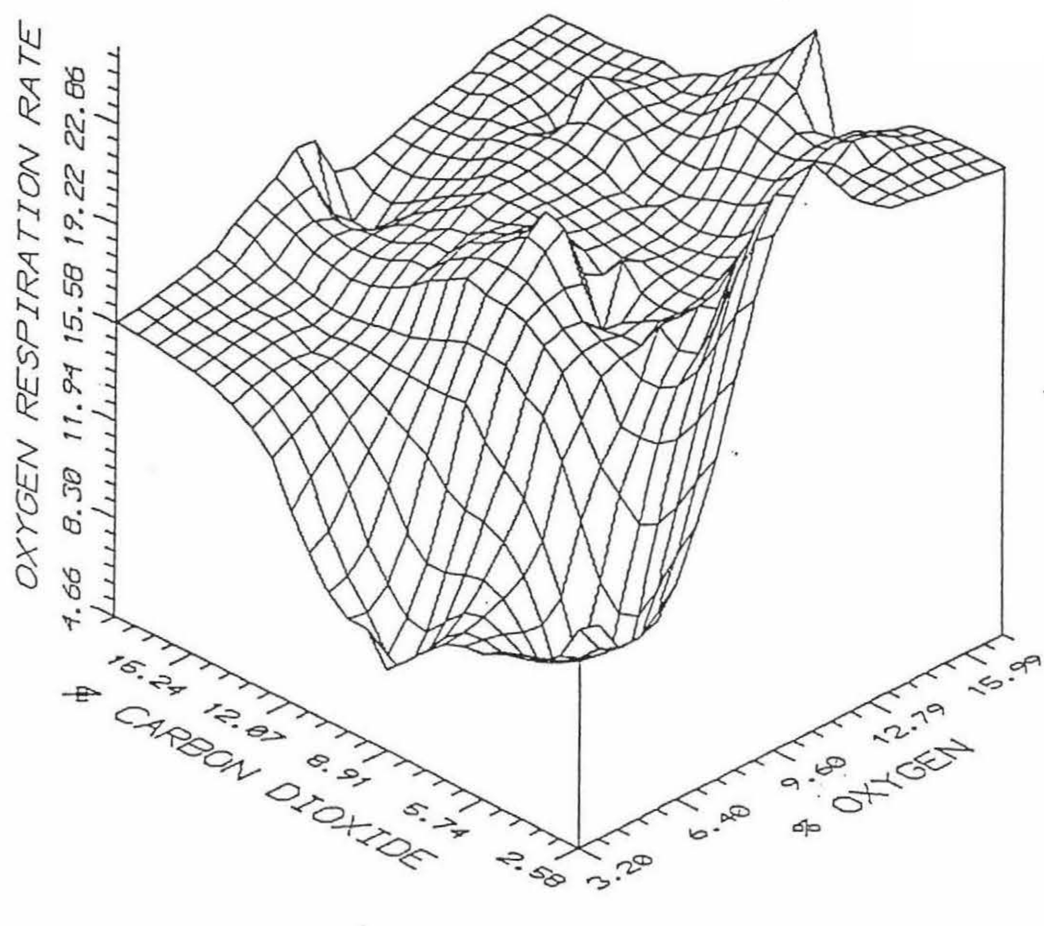


Figure A3.3 Carbon Dioxide Respiration Rate Topographical Map Drawn by "Surfer" (Carbon Dioxide Repiration Rates (x 10^{-8} moles.kg^{-1}.s^{-1}))

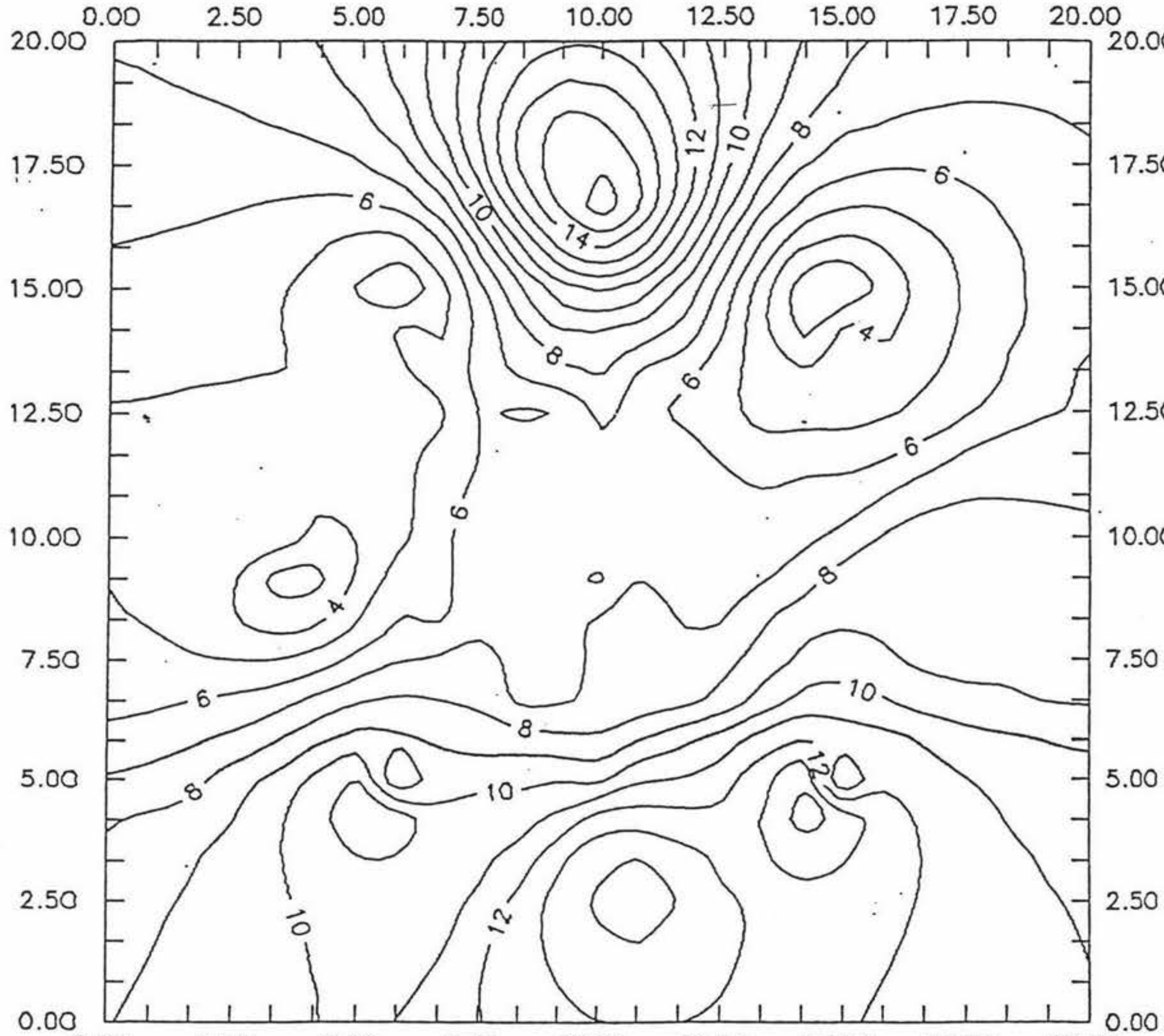
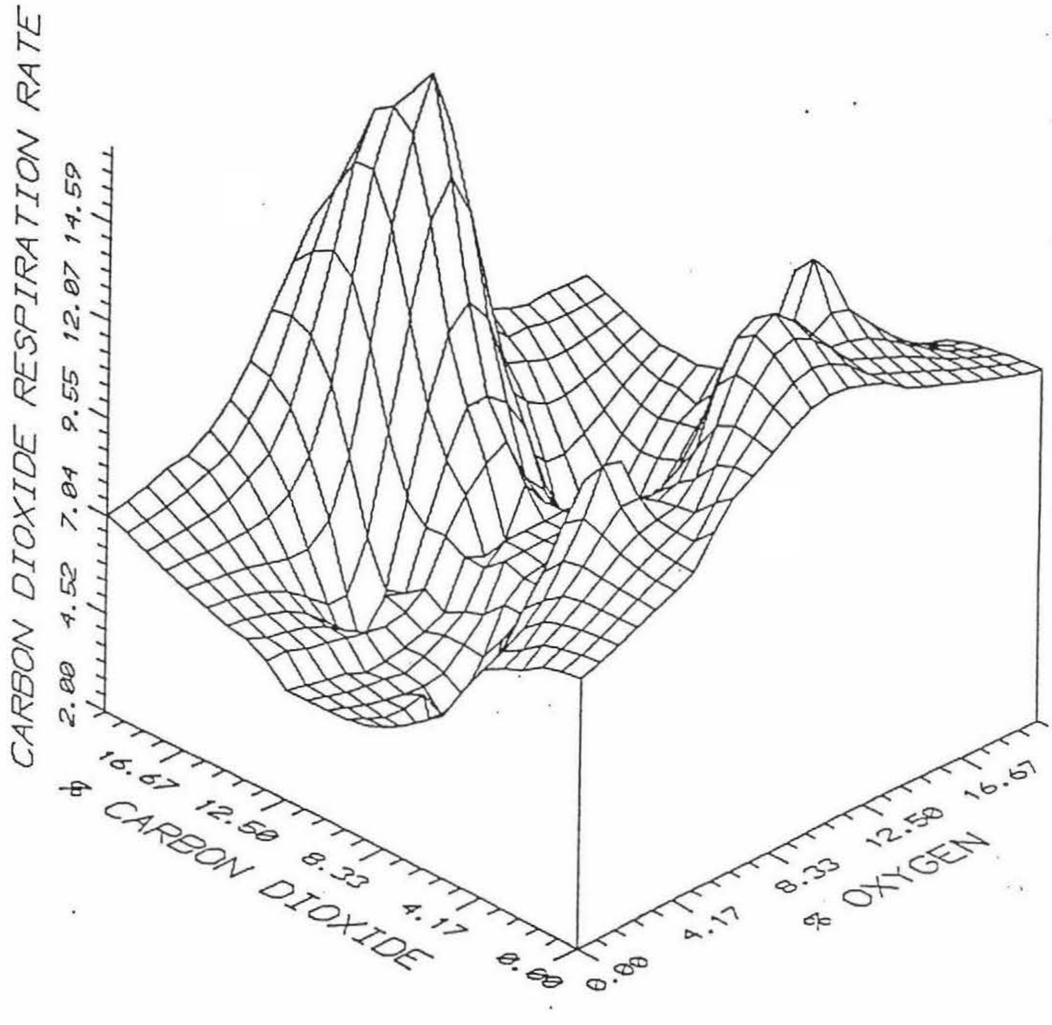


Figure A3.4 Carbon Dioxide Respiration Rate 3-Dimensional Diagrams Drawn by "Surfer"

(Carbon Dioxide Repiration Rates (x 10^{-8} moles.kg^{-1}.s^{-1}))



APPENDIX 4: Packaging Design "Quattro Pro" Spreadsheet.

Area m2	film Thickness m		Garlic Weight	Permeability m2/s	Garlic Repiration	Gas Composition start	finish	
POLYBUTYLENE		100mm x 200mm			O2			
0.04	7E-05	0.000698	0.399	1.94E-12	5E-08	21	3	oxygen
					CO2			
0.04	7E-05	0.000701	0.401	3.88E-12	5E-08	0.033	9	carbon dioxide
LDPE	200mm X 200mm							
0.08	7E-05	0.000589	0.674	2.39E-12	7.3E-08	21	3	
					CO2			
0.08	7E-05	0.000584	0.668	1.045E-11	9E-08	0.033	5	

APPENDIX 5: Spreadsheet Used to Calculate the Respiration Rates from a Dynamic System.

P1			6.18E-13	OXYGEN m2/s	T1		298	-0.000
V			0.000615	m3			275	4823
A			0.06	m2			8.3143	-1.354
b			3E-05	m			-25.406	
W		day	0.1	kg		P2	9.3E-12	
c		0	9.172	9.172	9.172	9.172	9.172	9.172
	1	2	7.882	7.794	7.794	7.619	7.619	7.094
	3	4	7.0938	6.481	7.094	7.225	6.875	5.868
	5.5	7	4.904	4.904	5.167	6.393	5.561	4.510
	8.5	10	1.6114	3.240	1.528	2.058	3.630	2.216
	12	14	1.3137	0.920	1.152	1.160	2.176	1.629
	16	18	1.121		0.906	1.642		1.708
	21	24	0.968		0.959			1.020
sec/day			86400					

O_2 Content of Packs moles/m^3

day						
0	9.172	9.172	9.172	9.172	9.172	9.172
2	5.911	6.349	5.868	5.605	4.817	6.393
4	5.561	5.911	5.780	5.386	4.685	6.174
7	5.649	5.255	5.780	4.685	3.670	5.605
10	5.342	5.298	5.955	4.423	3.380	5.298
14	5.561	5.036	6.393	3.980	2.439	6.481
24	6.043	3.556	4.291	2.124	2.106	3.324

Sliced Onion#4		#1	#3	7	16	17		Whole	4	10	11	19	21	22
oxygen								Sliced Onion						
respiration	-3E-08	-5E-08	-5E-08	-6E-08	-5.5E-08	-7E-08	-5E-08		-1E-07	-1E-07	-1E-07	-1E-07	-2E-07	-1E-07
moles/kg.s	-2.8E-08	-5E-08	-2E-08	-1E-08	-2.6E-08	-4E-08	-3E-08		-1E-08	-2E-08	-3E-09	-8E-09	-5E-09	-8E-09
	-5.2E-08	-4E-08	-5E-08	-2E-08	-3.1E-08	-3E-08	-4E-08		2.1E-09	-2E-08	1.3E-12	-2E-08	-2E-08	-1E-08
	-7.8E-08	-4E-08	-9E-08	-1E-07	-4.6E-08	-5E-08	-7E-08		-7E-09	1E-09	4.2E-09	-6E-09	-7E-09	-7E-09
	-5.3E-09	-4E-08	-7E-09	-2E-08	-2.6E-08	-1E-08	-2E-08		3.9E-09	-5E-09	7.8E-09	-8E-09	-2E-08	?.1E-08
	-3.4E-09		-4E-09	8.6E-09		1.4E-09	5.5E-10		3.4E-09	-1E-08	-1E-08	-1E-08	-2E-09	-2E-08
	-1.8E-09		6.3E-10			-8E-09	-3E-09							

OXYGEN CONTENT of PACKS %v/v

	2.283689	20.946	20.946	20.946	20.946	20.946	20.946		20.946	20.946	20.946	20.946	20.946	20.946
18	7.881982	17.8	17.8	17.4	17.4	16.2	17.32		13.5	14.5	13.4	12.8	11	14.6
16.2		14.8	16.2	16.5	15.7	13.4	15.32		12.7	13.5	13.2	12.3	10.7	14.1
11.2		11.2	11.8	14.6	12.7	10.3	12.12		12.9	12	13.2	10.7	8.38	12.8
3.68		7.4	3.49	4.7	8.29	5.06	5.788		12.2	12.1	13.6	10.1	7.72	12.1
3		2.1	2.63	2.65	4.97	3.72	3.214		12.7	11.5	14.6	9.09	5.57	14.8
2.56		2.07	2.07	3.75		3.9	2.9475		13.8	8.12	9.8	4.85	4.81	7.59
2.21	2.21		2.19			2.33	2.2433							

CARBON DIOXIDE RESPIRATION

CO_2 Content of Packs: Moles/m^3

c	0	0.014	0.014	0.014	0.014	0.014	0.014	0	0.014	0.014	0.014	0.014	0.014	0.014
x	2	0.858	1.055	0.928	0.963	1.012	1.112	2	1.695	1.594	1.594	1.808	2.168	1.581
	4	1.301	1.493	1.209	1.165	1.292	1.336	4	1.519	1.406	1.423	1.585	1.765	1.331
	7	2.150	2.382	2.089	1.808	2.006	1.773	7	1.511	1.484	1.283	1.756	1.905	1.371
	10	2.522	2.601	2.614	2.789	2.570	2.076	10	1.296	1.187	1.103	1.533	1.830	1.230
	14	2.400	2.767	2.575	2.645	2.763	2.224	14	1.493	1.384	1.134	1.844	2.408	1.344
	18	2.330		2.487	2.518		2.211	24	1.576	2.032	1.918	2.912	3.240	2.207
	24	3.048		3.332			3.109							
sec/day		86400												

Sliced Onion#4		#1	#3	7	16	17		Whole	4	10	11	19	21	22
carbon dioxide perm								Sliced Onion						
3E-12								AVERAGE						
	-1.6E-08	-2E-08	-1E-08	-7E-09	-1E-08	-8E-09	-1E-08		6.2E-09	6.7E-09	6.1E-09	7.9E-09	1.4E-08	8.9E-09
	-2E-08	-2E-08	-2E-08	-2E-08	-1.7E-08	-1E-08	-2E-08		1.9E-10	-2E-09	3.3E-09	-4E-09	-3E-09	-9E-10
	-8.8E-09	-5E-09	-1E-08	-2E-08	-1.3E-08	-7E-09	-1E-08		5.1E-09	7.1E-09	4.2E-09	5.3E-09	1.8E-09	3.3E-09
	2.17E-09	-3E-09	6.9E-10	2.6E-09	-3.4E-09	-3E-09	-6E-10		-4E-09	-4E-09	-6E-10	-6E-09	-1E-08	-2E-09
	1.24E-09	4.9E-08	1.5E-09	2.2E-09	4.9E-08	2.2E-10	1.7E-08		-1E-09	-1E-08	-1E-08	-2E-08	-1E-08	-2E-08
	-8.5E-09	-2E-11	-1E-08	3E-08	-1.6E-11	-1E-08	1E-10		1.9E-08	2.4E-08	2.3E-08	3.5E-08	3.8E-08	2.6E-08

CO_2 Content of Packs: ~~moles~~ %v/v

2.284	0.033	0.033	0.033	0.033	0.033	0.033	0.033		0.033	0.033	0.033	0.033	0.033	0.033
	1.96	2.41	2.12	2.2	2.31	2.54	2.2567		3.87	3.64	3.64	4.13	4.95	3.61
	2.97	3.41	2.76	2.66	2.95	3.05	2.9667		3.47	3.21	3.25	3.62	4.03	3.04
	4.91	5.44	4.77	4.13	4.58	4.05	4.6467		3.45	3.39	2.93	4.01	4.35	3.13
	5.76	5.94	5.97	6.37	5.87	4.74	5.775		2.96	2.71	2.52	3.5	4.18	2.81
	5.48	6.32	5.88	6.04	6.31	5.08	5.8517		3.41	3.16	2.59	4.21	5.5	3.07
	5.32	5.74	5.68	5.75	6.46	5.05	5.6667		3.6	4.64	4.38	6.65	7.4	5.04
	6.96	7.8	7.61	8.02	8.55	7.1	7.6733							

RESPIRATION mg/kg.hr													
convert	-2.50	-2.47	-1.58	-1.14	-1.58	-1.26	-1.75	0.72	0.77	0.70	0.91	1.65	1.02
factor2E+08	-3.19	-3.34	-3.31	-2.42	-2.68	-1.65	-2.77	0.02	-0.22	0.38	-0.47	-0.38	-0.11
	-1.40	-0.82	-1.98	-3.69	-2.12	-1.14	-1.86	0.58	0.81	0.49	0.61	0.20	0.38
	0.34	-0.47	0.11	0.41	-0.54	-0.42	-0.10	-0.41	-0.41	-0.06	-0.64	-1.19	-0.23
	0.20	7.80	0.25	0.36	7.79	0.04	2.74	-0.17	-1.33	-1.61	-2.19	-1.71	-1.77
	-1.35	-0.00	-1.59	4.73	-0.00	-1.69	0.02	2.15	2.77	2.62	3.98	4.43	3.01

APPENDIX 6: Printout from the RSM Programme on the Sliced Onion Respiration Models. (All Respiration Rates (x 10^{-8}) moles.kg^{-1}.s^{-1}, O_2 and CO_2 in % V/V)

VARIABLES AND LEVELS

	-1.6	-1.0	0.0	1.0	1.6
X1 = oxygen					18.00
X2 = carbon dioxide	2.00	5.00	10.00	15.00	18.00
X3 = slice thickness					18.00
	2.00	5.00	10.00	15.00	
	2.00	5.00	10.00	15.00	

RESPONSES

Y1 = oxygen respiration

Y2 = carbon dioxide respiration

TAYLOR EXPANSION EQUATION AND CALCULATED BETA COEFFICIENTS USING CODED VALUES FOR X'S

Yi = B0 *X1 + B2 *X2 + B3 *X3 + B11*X1^2 + B22*X2^2 + B33*X3^2
+ B12*X1*X2 + B13*X1*X3 + B23*X2*X3

	Y1	Y2
B0 =	4.956	2.172
B1 =	-0.075	-0.295
B2 =	-0.501	-0.857
B3 =	-0.107	-0.240
B11 =	-0.236	-0.279
B22 =	-0.420	-0.277
B33 =	-0.334	-0.084
B12 =	0.211	-0.126
B13 =	0.076	0.217
B23 =	-0.441	0.209

CALCULATED VERSUS
OBSERVED RESPONSE VALUES
FOR YOUR EXPERIMENTAL
RUNS

	Y1		Y2	
	CA	OB	CA	OB
1	4.0727	3.8400	0.0680	0.0000
2	4.9561	5.6000	2.1723	2.9900
3	4.4708	3.7300	1.9283	1.0200
4	4.9561	4.1000	2.1723	1.1500
5	4.9561	5.0400	2.1723	1.2900
6	4.6833	5.2400	2.8333	2.5000
7	5.0115	5.0600	1.8907	2.2300
8	4.2313	5.2600	0.9856	1.4700
9	4.4952	4.6400	3.2239	3.8100
10	3.9524	3.7600	1.3422	1.2800
11	4.9561	3.7600	2.1723	3.3200
12	3.0789	2.8100	0.0906	0.0000
13	3.9301	3.3600	1.5724	0.9800
14	2.7037	3.7500	0.8465	1.6400
15	3.1290	2.8000	0.4399	0.1250
16	3.7705	2.5400	2.4522	1.9300
17	4.9561	5.1800	2.1723	2.2400
18	4.5918	4.6000	1.9865	2.3200
19	4.9561	5.9300	2.1723	2.2300
20	4.2721	5.1300	2.3414	2.5100

Y1 - Oxygen Respiration

CONFIDENCE STATEMENTS

Standard Error = .9729236

Standard Deviation of Replicates = .8466195
This Equals 17.08 Percent of the Centerpoint Value

Multiple Correlation Coefficient = .7057414

49.79 Percent of the Variation is Explained by the Model

18.67 Percent by First Order (Linear) Effects
20.73 Percent by Second Order (Quadratic) Effects
10.39 Percent by Interaction Effects

19 Percent of the Variation is Experimental Error

31.21 Percent of the Variation is Unexplained
by Either the Model or the Experimental Error

ANALYSIS OF VARIANCE

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Calculated	F Table (95%)
Regression					
First order	3.52	3	1.17	1.64	5.41
Second Order	3.91	3	1.30	1.82	5.41
Interaction	1.96	3	0.65	0.91	5.41
Lack of Fit	5.88	5	1.18	1.64	5.05
Exper. Error	3.58	5	0.72		
Total	18.86	19	0.99		

Y2 - Carbon Dioxide Respiration

CONFIDENCE STATEMENTS

Standard Error = .8339849

Standard Deviation of Replicates = .8737654
This Equals 40.22 Percent of the Centrepoint Value

Multiple Correlation Coefficient = .8198839

67.21 Percent of the Variation is Explained by the Model

54.35 Percent by First Order (Linear) Effects
8.84 Percent by Second Order (Quadratic) Effects
4.02 Percent by Interaction Effects

17.99 Percent of the Variation is Experimental Error

14.8 Percent of the Variation is Unexplained
by Either the Model or the Experimental Error

ANALYSIS OF VARIANCE

Source	Sum of Squares	Degrees of Freedom	Mean Square	F Calculated	F Table (95%)
Regression					
First order	11.53	3	3.84	5.04	5.41
Second Order	1.88	3	0.63	0.82	5.41
Interaction	0.85	3	0.28	0.37	5.41
Lack of Fit	3.14	5	0.63	0.82	5.05
Exper. Error	3.82	5	0.76		
Total	21.22	19	1.12		

Figure A6.1 XY Plot of the change in O_2 respiration rate at three slice thicknesses, 5mm, 10mm and 15mm at 2% CO_2.

Slice Thickness L = 5mm, M = 10mm, H = 15mm.
Oxygen Respiration Rate (x 10^{-8} moles.kg^{-1}.s^{-1})
Carbon dioxide level is constant at 2% v/v.

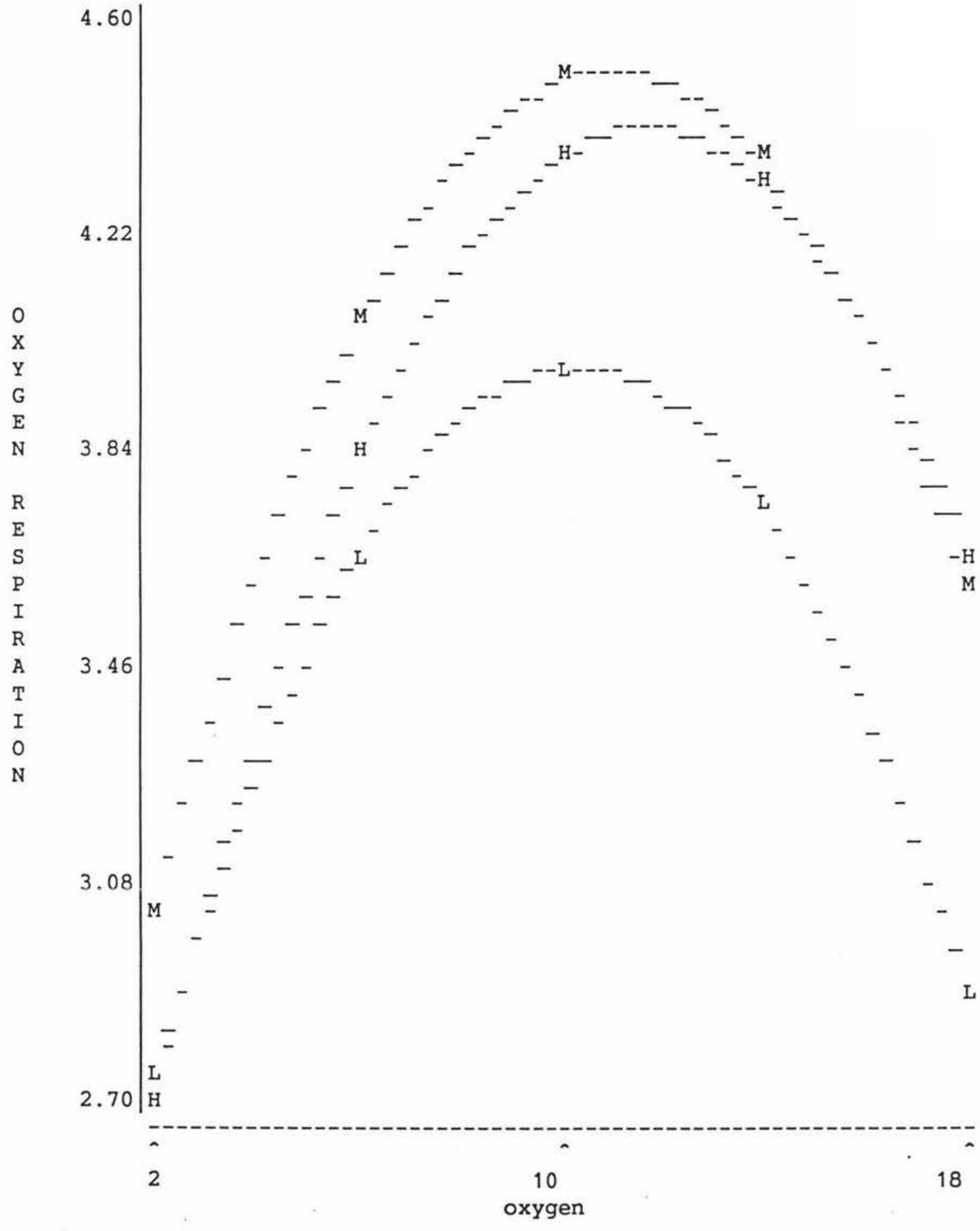
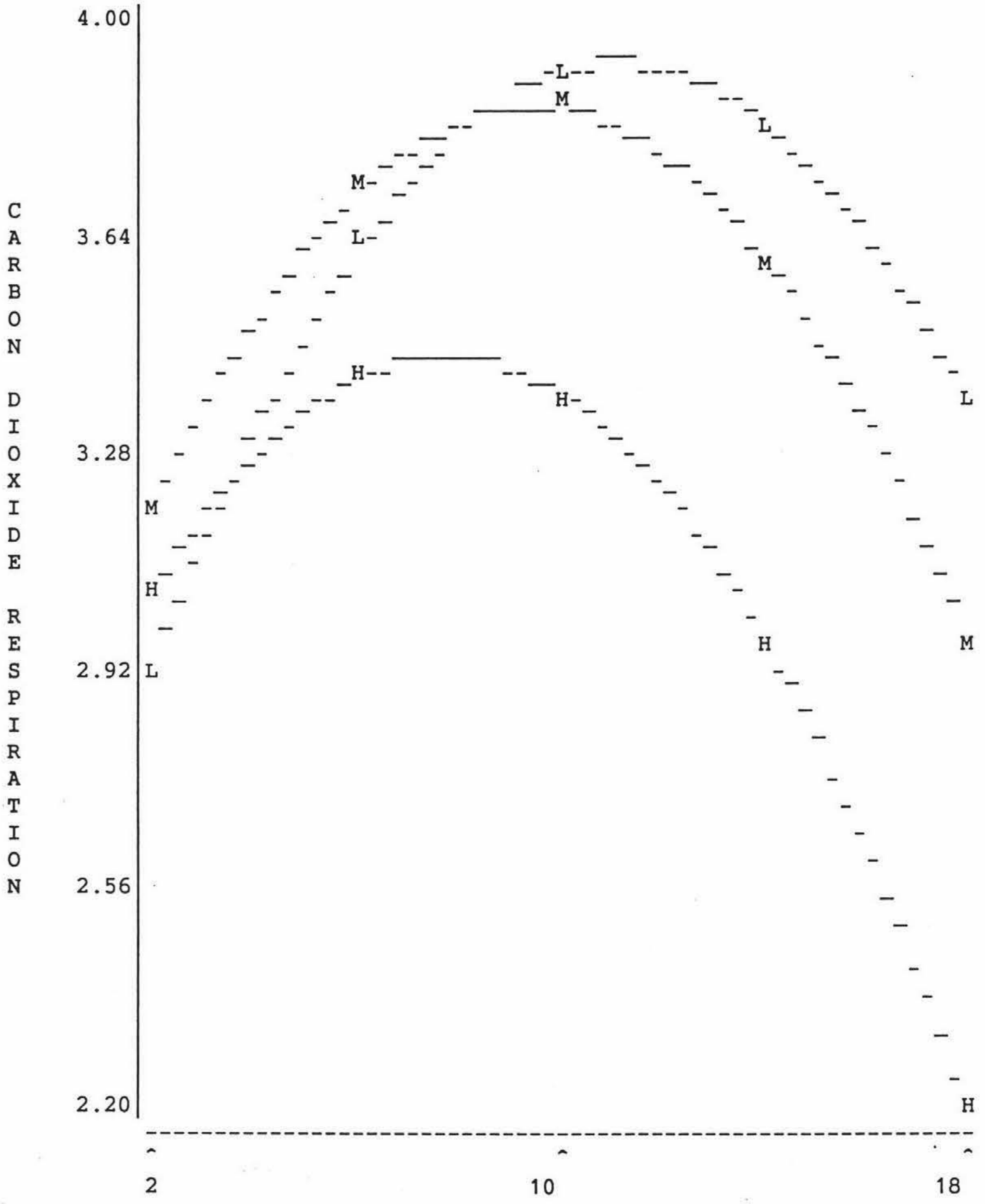


Figure A6.2 XY Plot of the change in CO_2 respiration rate at three slice thicknesses, 5mm, 10mm and 15mm at 2% CO_2.

Slice Thickness L = 5mm, M = 10mm, H = 15mm.
Carbon Dioxide Respiration Rate (x 10^{-8} moles.kg^{-1}.s^{-1})
Carbon dioxide level is constant at 2% v/v.



gure A6.3 XY Plot of the change in O_2 respiration rate at three slice thicknesses, 5mm, 10mm and 15mm at 4% CO_2.

Slice Thickness L = 5mm, M = 10mm, H = 15mm.
Oxygen Respiration Rate (x 10^{-8} moles.kg^{-1}.s^{-1})
Carbon Dioxide level is constant at 4% v/v.

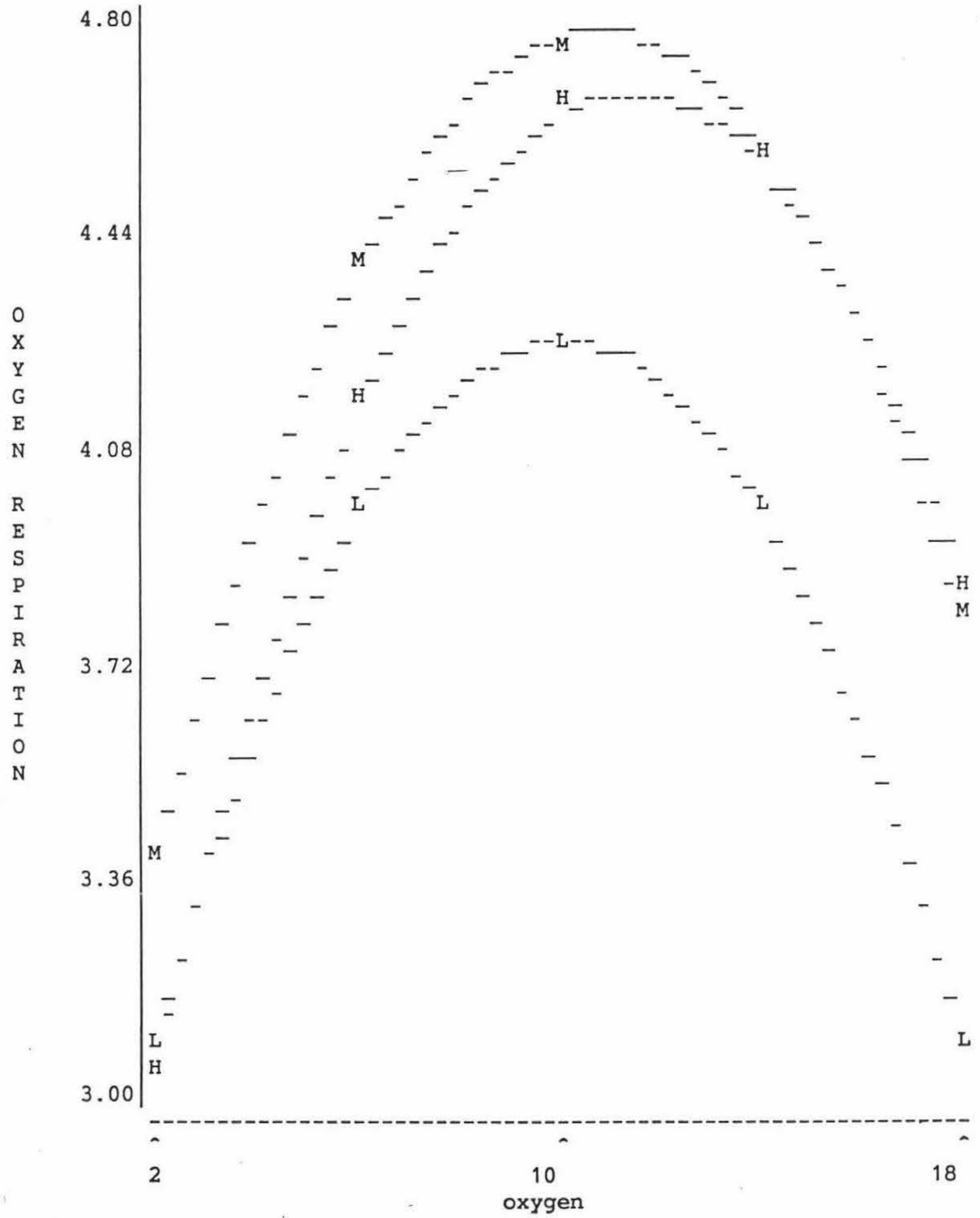


Figure A6.4 XY Plot of the change in CO_2 respiration rate at three slice thicknesses, 5mm, 10mm and 15mm at 4% CO_2.

Slice Thickness L = 5mm, M = 10mm, H = 15mm.
Carbon Dioxide Respiration Rate (x 10^{-8} moles.$kg^{-1}.s^{-1}$)
Carbon dioxide level is constant at 4% v/v.

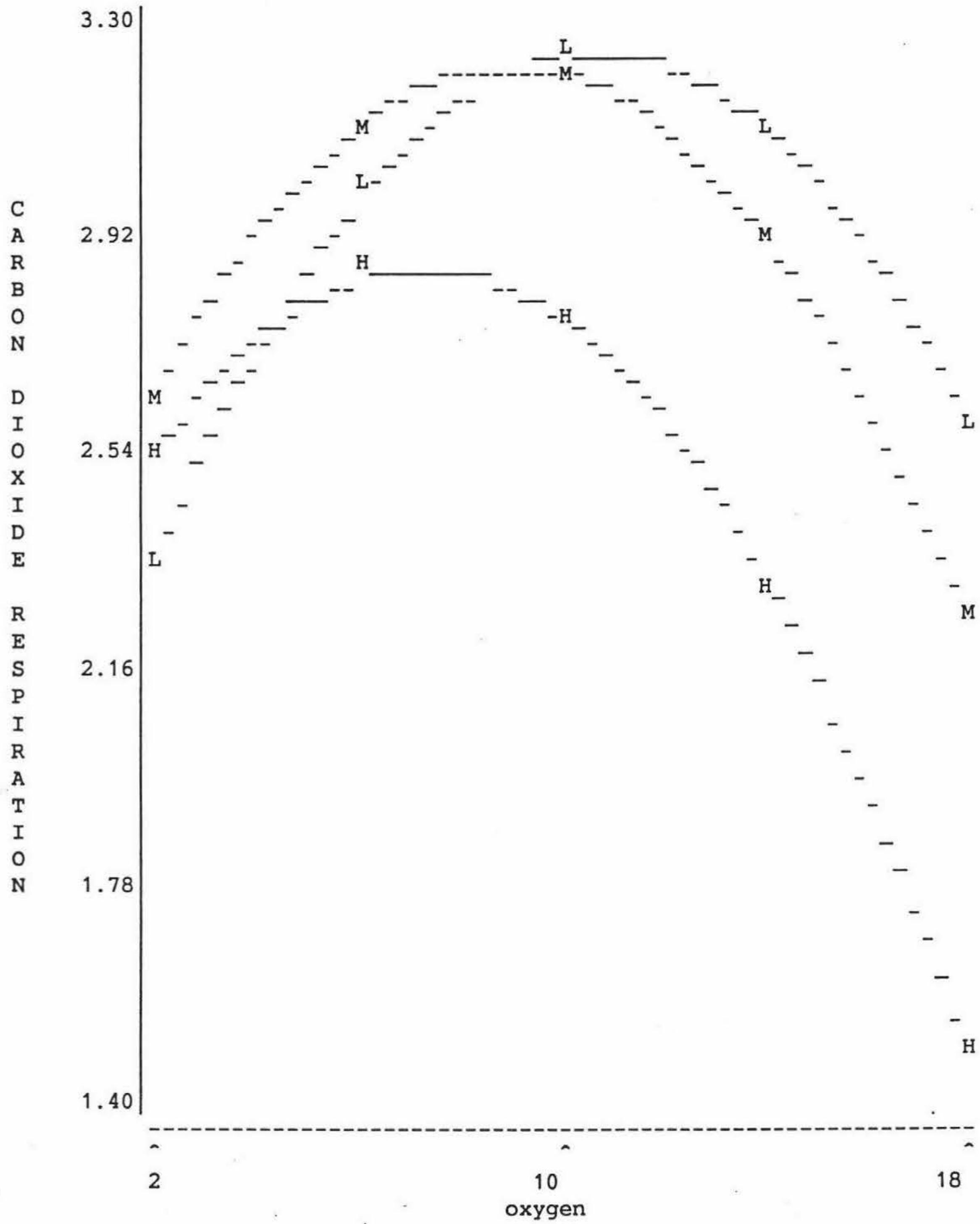
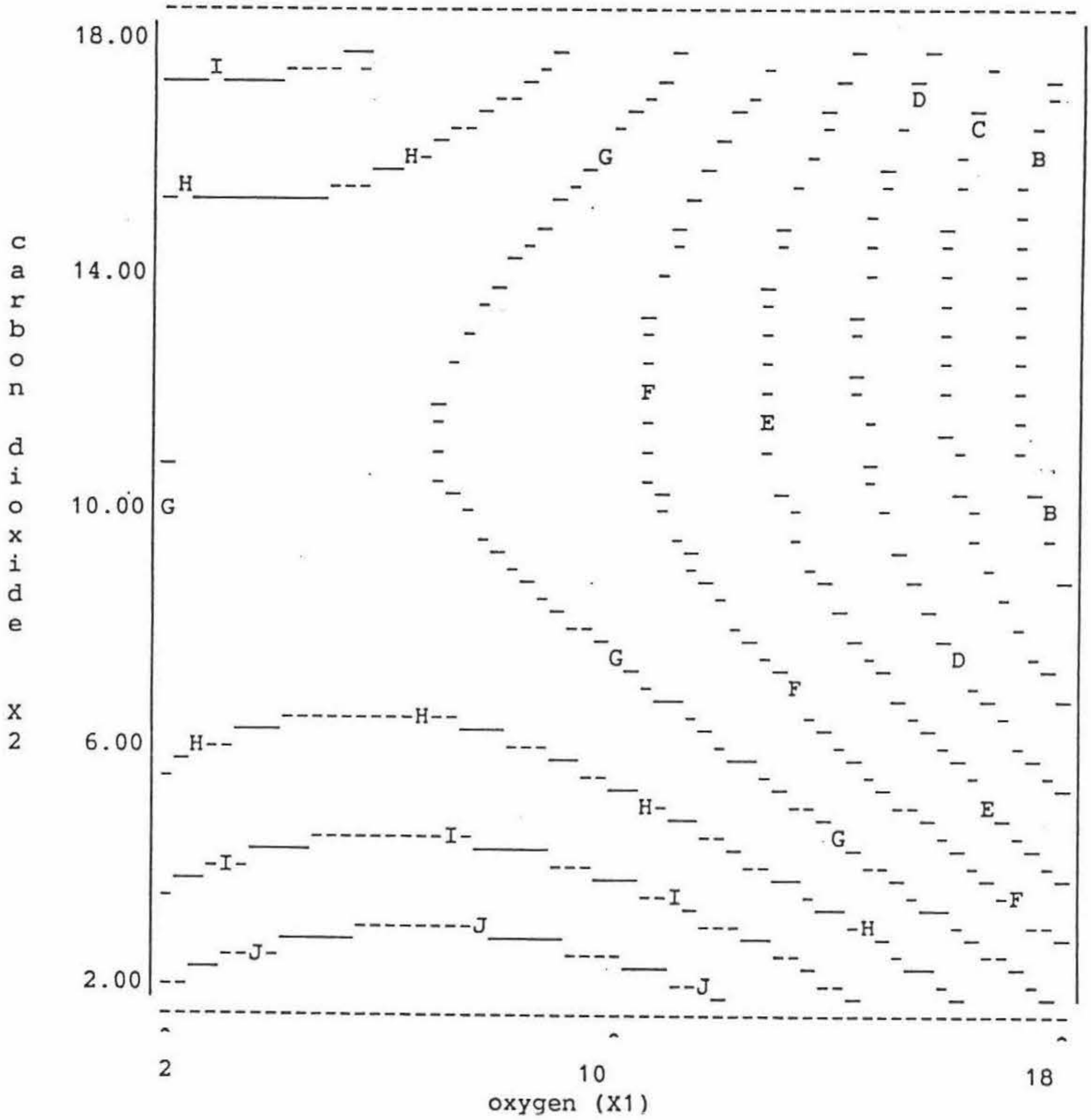


Figure A6.5 RSM generated Topographical Map of the CO_2 model at an onion slice thickness of 18mm.

X-axis is percent oxygen v/v and the Y-axis is percent carbon dioxide v/v
Slice Thickness is held constant at 18mm.

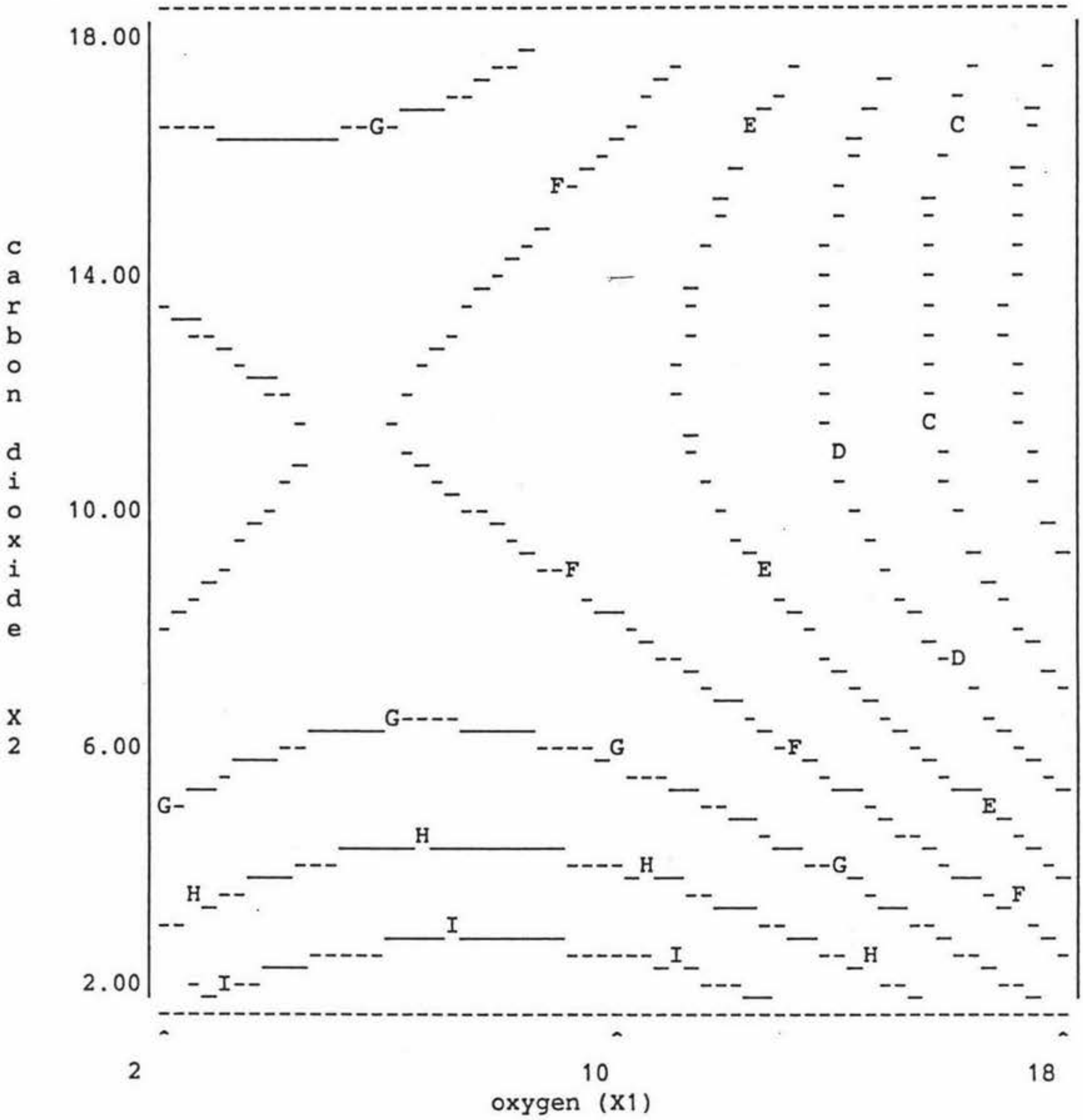


Where lines on the face of the map are the carbon dioxide respiration rate in moles.$kg^{-1}.s^{-1}$ at the following levels:

A	=	-0.7×10^{-8}	G	=	1.6×10^{-8}
B	=	-0.3×10^{-8}	H	=	2.0×10^{-8}
C	=	0.1×10^{-8}	I	=	2.3×10^{-8}
D	=	0.4×10^{-8}	J	=	2.7×10^{-8}
E	=	0.8×10^{-8}	K	=	3.1×10^{-8}
F	=	1.2×10^{-8}	L	=	3.5×10^{-8}

Figure A6.6 RSM generated Topographical Map of the CO_2 model at an onion slice thickness of 15mm.

X-axis is percent oxygen v/v and the Y-axis is percent carbon dioxide v/v
Slice Thickness is held constant at 15mm.



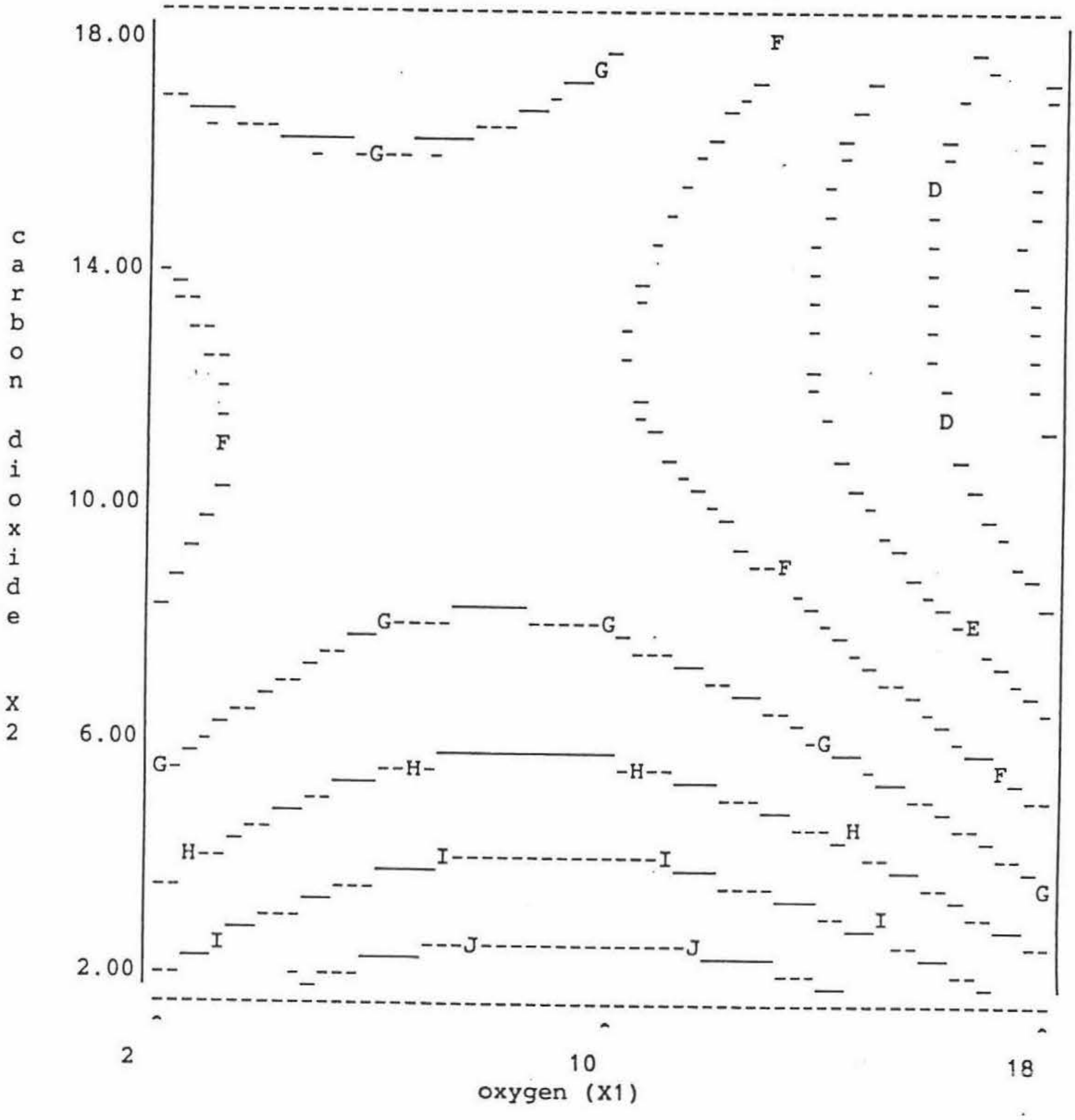
Where lines on the face of the map are the carbon dioxide respiration rate in moles.kg^{-1}.s^{-1} at the following levels:

A	=	-0.7×10^{-8}	G	=	1.6×10^{-8}
B	=	-0.3×10^{-8}	H	=	2.0×10^{-8}
C	=	0.1×10^{-8}	I	=	2.3×10^{-8}
D	=	0.4×10^{-8}	J	=	2.7×10^{-8}
E	=	0.8×10^{-8}	K	=	3.1×10^{-8}
F	=	1.2×10^{-8}	L	=	3.5×10^{-8}

The X-axis is percent oxygen v/v and the Y-axis is percent carbon dioxide v/v

Figure A6.7 RSM generated Topographical Map of the CO_2 model at an onion slice thickness of 10mm.

X-axis is percent oxygen v/v and the Y-axis is percent carbon dioxide v/v
Slice Thickness is held constant at 10mm.

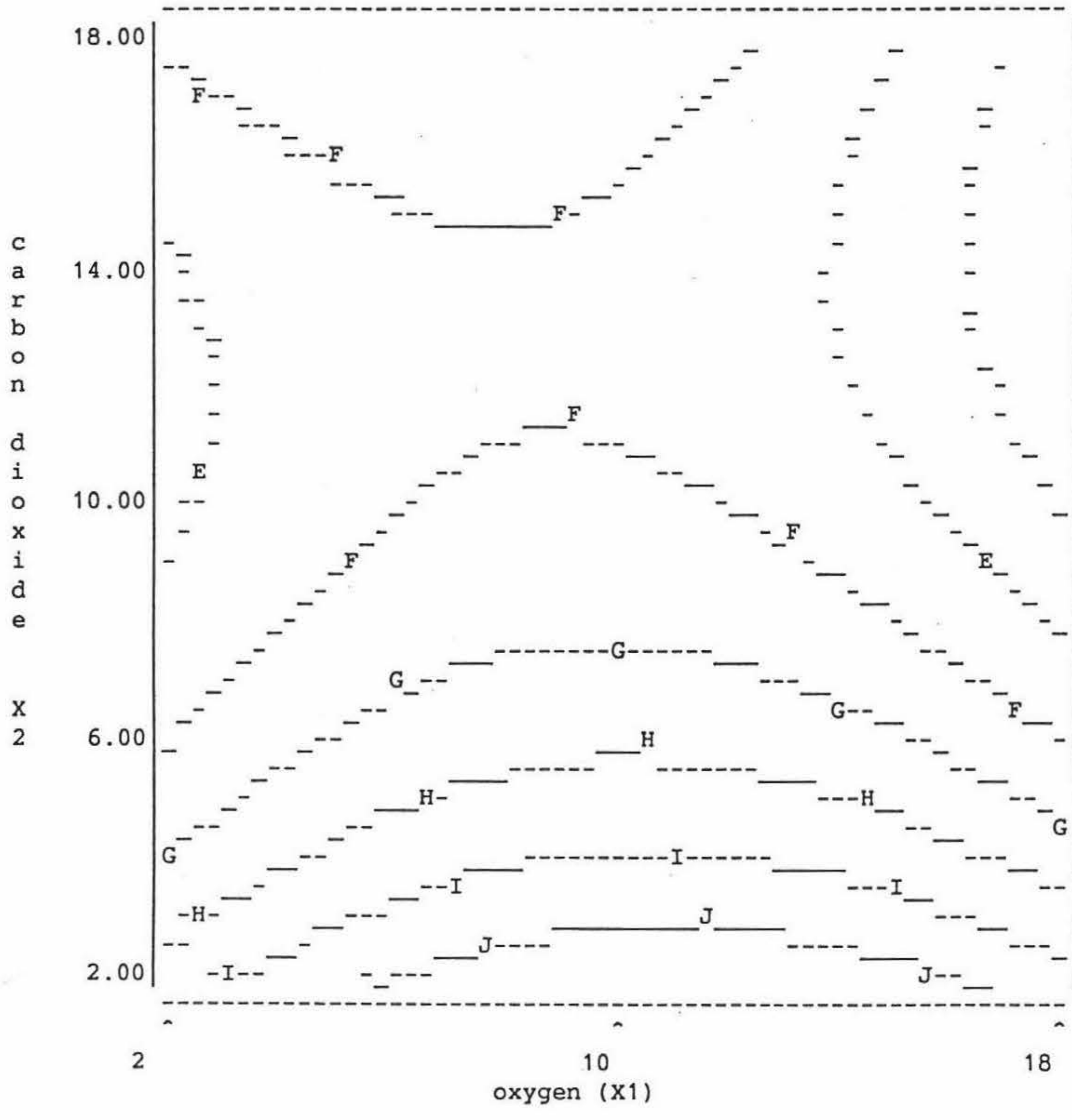


Where lines on the face of the map are the carbon dioxide respiration rate in moles.$kg^{-1}.s^{-1}$ at the following levels:

A	=	-0.7×10^{-8}	G	=	1.6×10^{-8}
B	=	-0.3×10^{-8}	H	=	2.0×10^{-8}
C	=	0.1×10^{-8}	I	=	2.3×10^{-8}
D	=	0.4×10^{-8}	J	=	2.7×10^{-8}
E	=	0.8×10^{-8}	K	=	3.1×10^{-8}
F	=	1.2×10^{-8}	L	=	3.5×10^{-8}

Figure A6.8 RSM generated Topographical Map of the CO_2 model at an onion slice thickness of 5mm.

X-axis is percent oxygen v/v and the Y-axis is percent carbon dioxide v/v
Slice Thickness is held constant at 5mm.

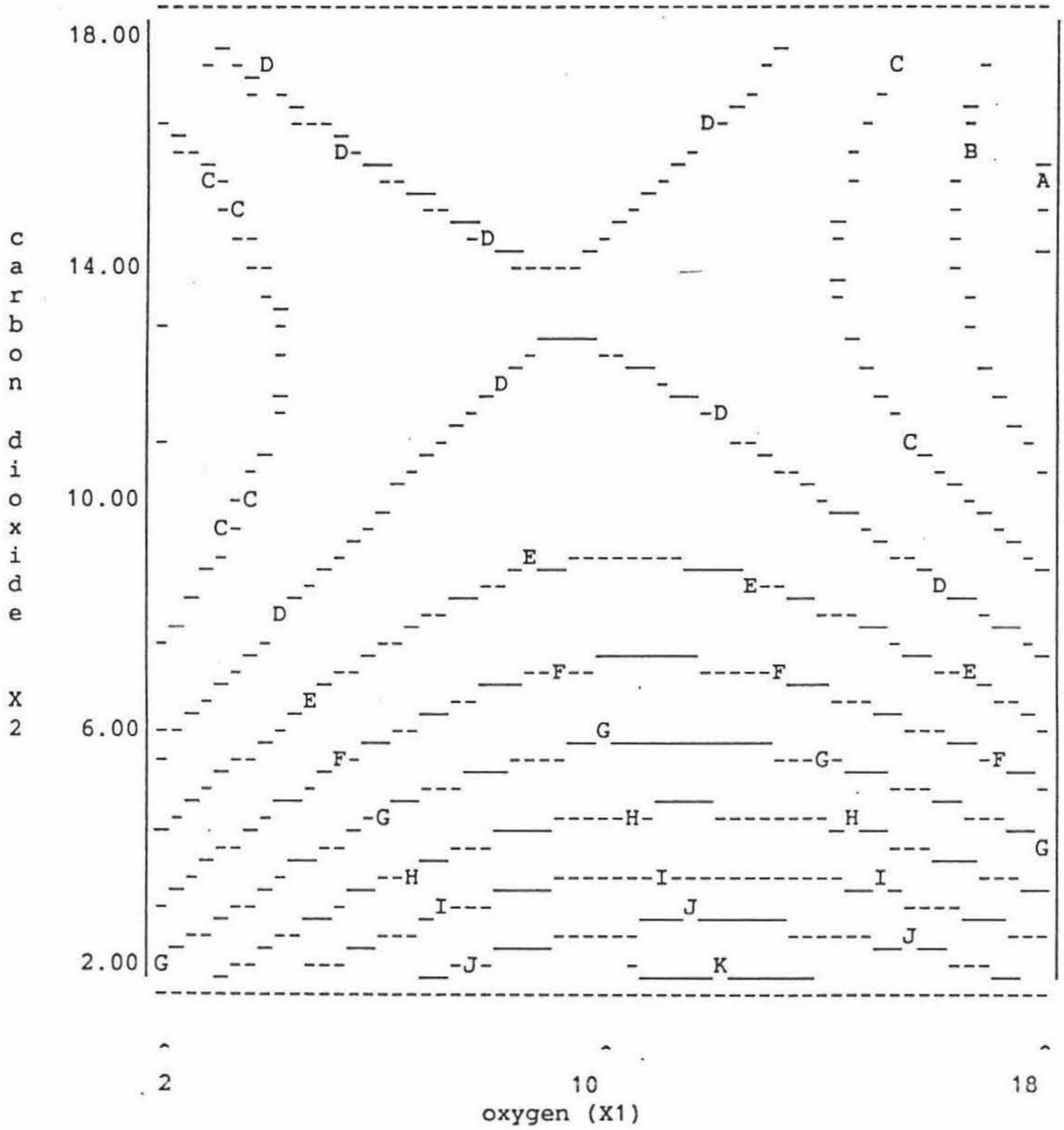


Where lines on the face of the map are the carbon dioxide respiration rate in moles.kg^{-1}.s^{-1} at the following levels:

A	=	-0.7 x 10^{-8}	G	=	1.6 x 10^{-8}
B	=	-0.3 x 10^{-8}	H	=	2.0 x 10^{-8}
C	=	0.1 x 10^{-8}	I	=	2.3 x 10^{-8}
D	=	0.4 x 10^{-8}	J	=	2.7 x 10^{-8}
E	=	0.8 x 10^{-8}	K	=	3.1 x 10^{-8}
F	=	1.2 x 10^{-8}	L	=	3.5 x 10^{-8}

Figure A6.9 RSM generated Topographical Map of the CO_2 model at an onion slice thickness of 2mm.

X-axis is percent oxygen v/v and the Y-axis is percent carbon dioxide v/v
Slice Thickness is held constant at 2mm.

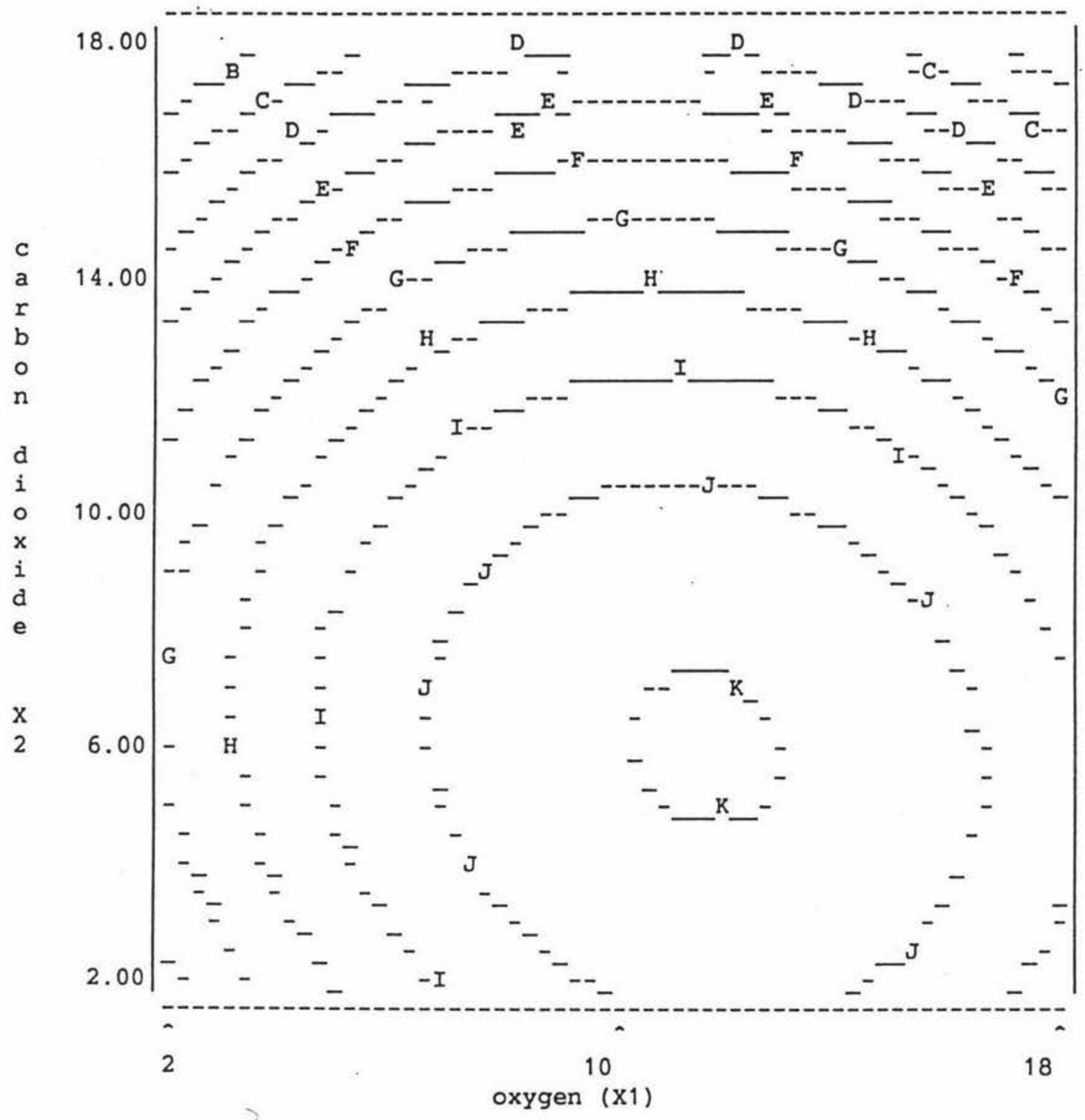


Where lines on the face of the map are the carbon dioxide respiration rate in $moles.kg^{-1}.s^{-1}$ at the following levels:

A	=	-0.7×10^{-8}	G	=	1.6×10^{-8}
B	=	-0.3×10^{-8}	H	=	2.0×10^{-8}
C	=	0.1×10^{-8}	I	=	2.3×10^{-8}
D	=	0.4×10^{-8}	J	=	2.7×10^{-8}
E	=	0.8×10^{-8}	K	=	3.1×10^{-8}
F	=	1.2×10^{-8}	L	=	3.5×10^{-8}

Figure A6.10 RSM generated Topographical Map of the O_2 model at an onion slice thickness of 18mm.

X-axis is percent oxygen v/v and the Y-axis is percent carbon dioxide v/v
Slice Thickness is held constant at 18mm.

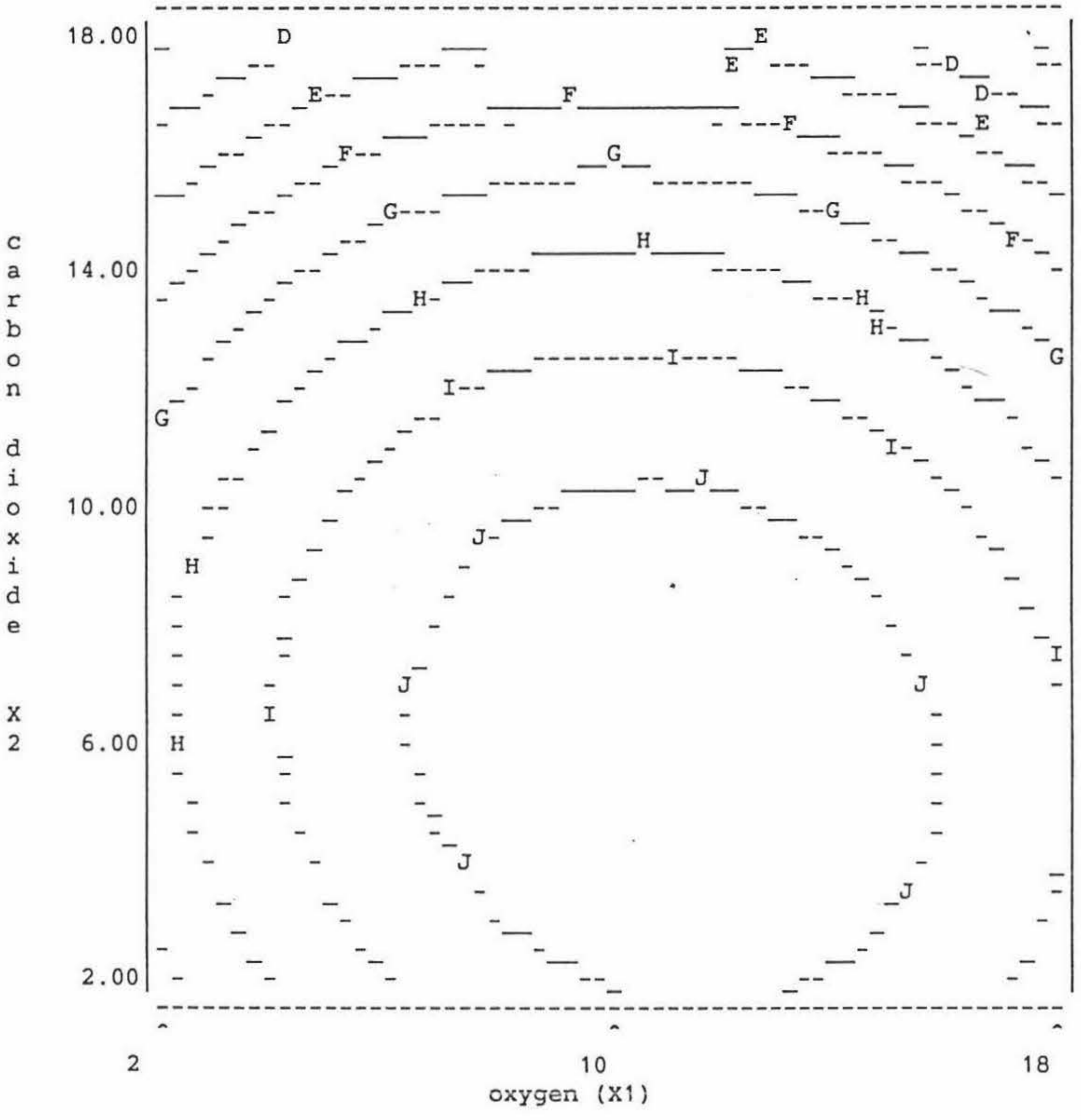


Where lines on the face of the map are the oxygen respiration rate in $moles.kg^{-1}.s^{-1}$ at the following levels:

A	=	0.3×10^{-8}	G	=	2.8×10^{-8}
B	=	0.7×10^{-8}	H	=	3.2×10^{-8}
C	=	1.1×10^{-8}	I	=	3.6×10^{-8}
D	=	1.5×10^{-8}	J	=	4.0×10^{-8}
E	=	1.9×10^{-8}	K	=	4.4×10^{-8}
F	=	2.4×10^{-8}	L	=	4.8×10^{-8}

Figure A6.11 RSM generated Topographical Map of the O_2 model at an onion slice thickness of 15mm.

X-axis is percent oxygen v/v and the Y-axis is percent carbon dioxide v/v
Slice Thickness is held constant at 15mm.

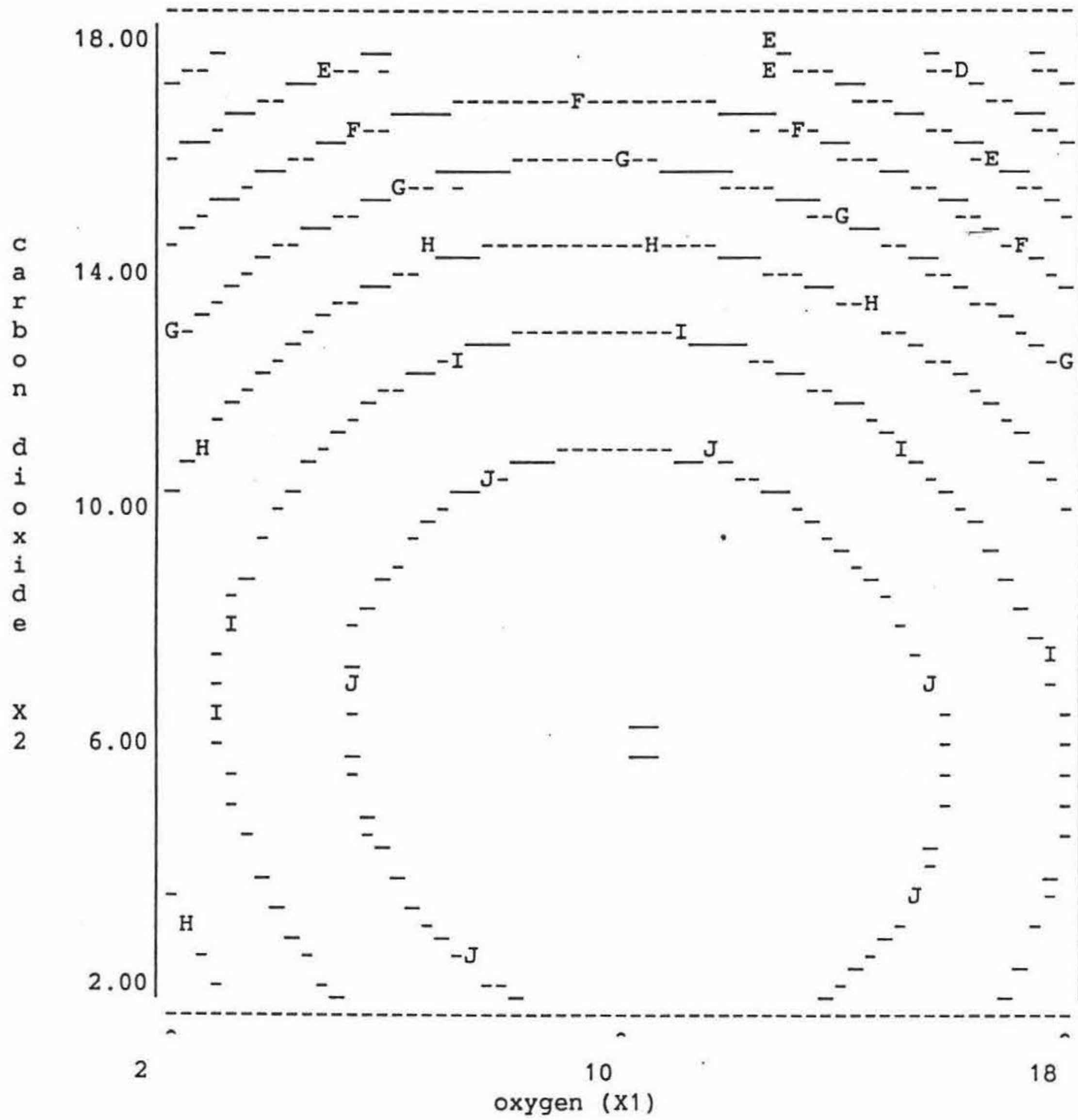


Where lines on the face of the map are the oxygen respiration rate in $moles.kg^{-1}.s^{-1}$ at the following levels:

A	=	0.3×10^{-8}	G	=	2.8×10^{-8}
B	=	0.7×10^{-8}	H	=	3.2×10^{-8}
C	=	1.1×10^{-8}	I	=	3.6×10^{-8}
D	=	1.5×10^{-8}	J	=	4.0×10^{-8}
E	=	1.9×10^{-8}	K	=	4.4×10^{-8}
F	=	2.4×10^{-8}	L	=	4.8×10^{-8}

Figure A6.12 RSM generated Topographical Map of the O_2 model at an onion slice thickness of 10mm.

X-axis is percent oxygen v/v and the Y-axis is percent carbon dioxide v/v
Slice Thickness is held constant at 10 mm.

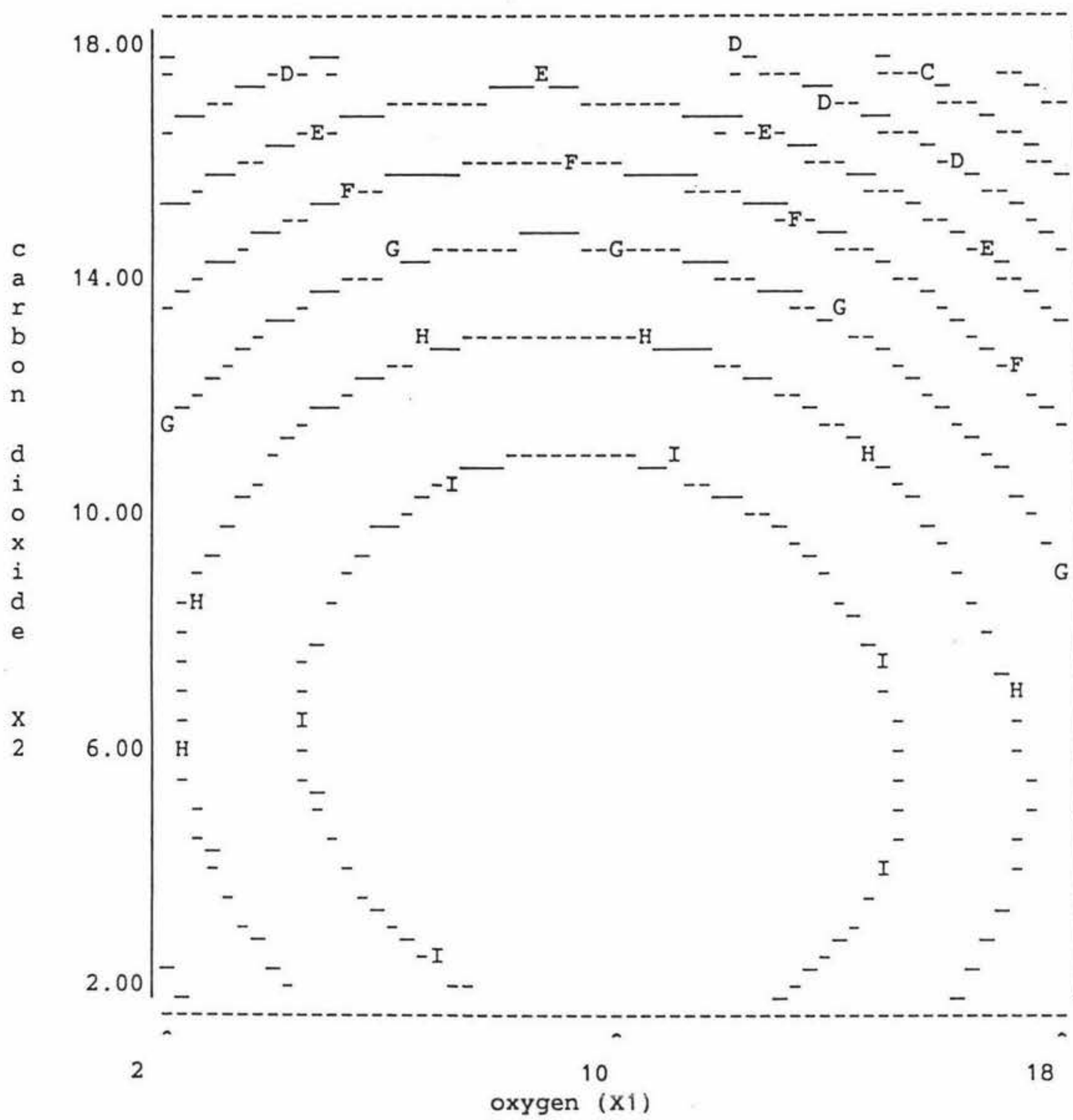


Where lines on the face of the map are the oxygen respiration rate in moles.kg^{-1}.s^{-1} at the following levels:

A	=	0.3×10^{-8}	G	=	2.8×10^{-8}
B	=	0.7×10^{-8}	H	=	3.2×10^{-8}
C	=	1.1×10^{-8}	I	=	3.6×10^{-8}
D	=	1.5×10^{-8}	J	=	4.0×10^{-8}
E	=	1.9×10^{-8}	K	=	4.4×10^{-8}
F	=	2.4×10^{-8}	L	=	4.8×10^{-8}

Figure A6.13 RSM generated Topographical Map of the O_2 model at an onion slice thickness of 5mm.

X-axis is percent oxygen v/v and the Y-axis is percent carbon dioxide v/v
Slice Thickness is held constant at 5mm.

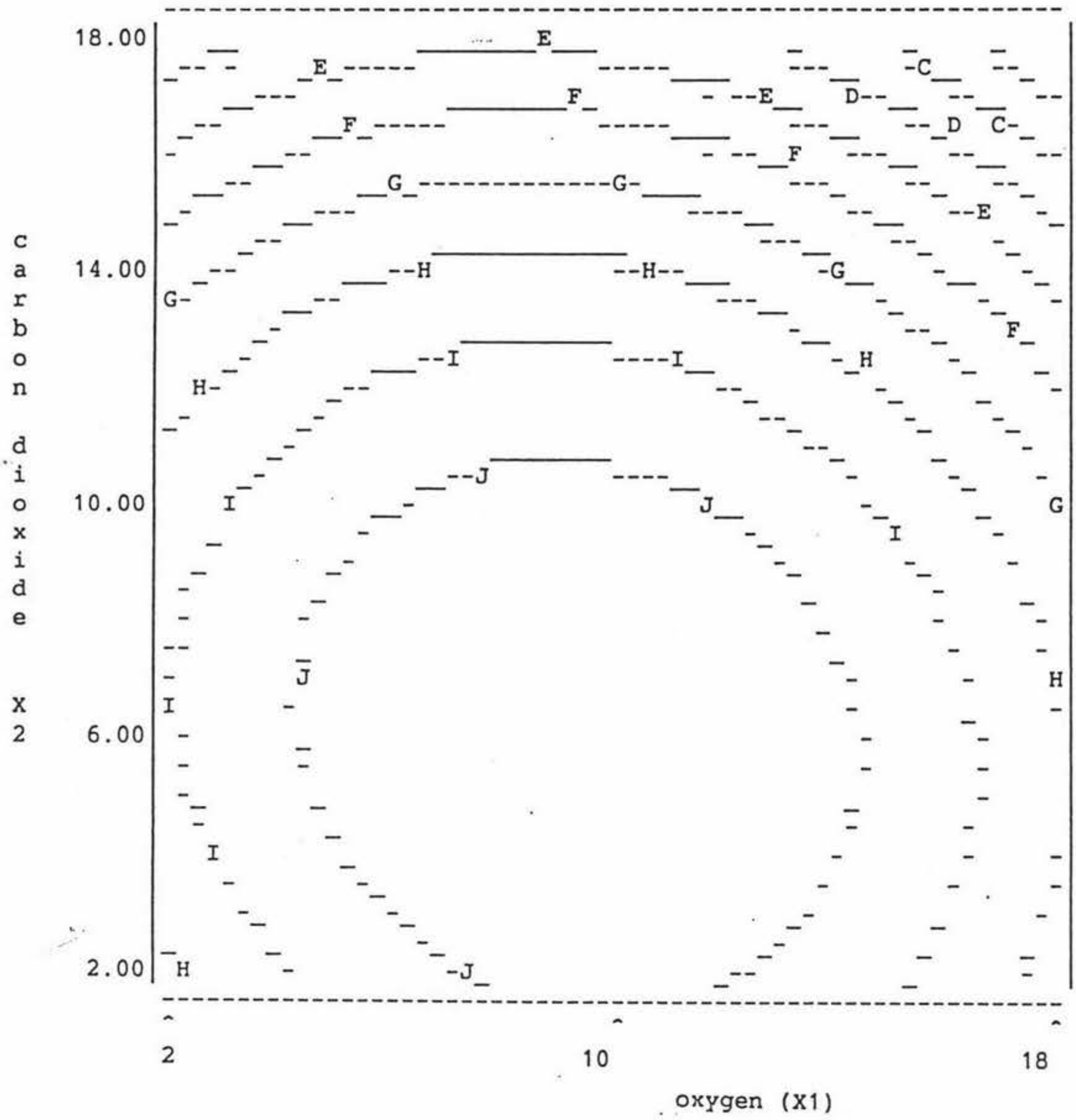


Where lines on the face of the map are the oxygen respiration rate in moles.$kg^{-1}.s^{-1}$ at the following levels:

A	=	0.3×10^{-8}	G	=	2.8×10^{-8}
B	=	0.7×10^{-8}	H	=	3.2×10^{-8}
C	=	1.1×10^{-8}	I	=	3.6×10^{-8}
D	=	1.5×10^{-8}	J	=	4.0×10^{-8}
E	=	1.9×10^{-8}	K	=	4.4×10^{-8}
F	=	2.4×10^{-8}	L	=	4.8×10^{-8}

Figure A6.14 RSM generated Topographical Map of the O_2 model at an onion slice thickness of 2mm.

X-axis is percent oxygen v/v and the Y-axis is percent carbon dioxide v/v
Slice Thickness is held constant at 2mm.



Where lines on the face of the map are the oxygen respiration rate in moles.$kg^{-1}.s^{-1}$ at the following levels:

A	=	0.3×10^{-8}	G	=	2.8×10^{-8}
B	=	0.7×10^{-8}	H	=	3.2×10^{-8}
C	=	1.1×10^{-8}	I	=	3.6×10^{-8}
D	=	1.5×10^{-8}	J	=	4.0×10^{-8}
E	=	1.9×10^{-8}	K	=	4.4×10^{-8}
F	=	2.4×10^{-8}	L	=	4.8×10^{-8}