INVESTIGATION OF THE POTENTIALLY DETRIMENTAL EFFECT OF CIPC APPLICATION ON THE PROCESSING QUALITY OF STORED POTATOES

Geraldine Dowd

BSc (Hons) University of Glasgow 1999

Thesis submitted for the degree of Doctor of Philosophy

January 2004

University of Glasgow
Environmental, Agricultural and Analytical Chemistry
Section, Department of Chemistry

Abstract

The provision of crops of a light fry colour, from store, is of the utmost importance to processors. Poor fry colour leads to rejection of crops on a quality basis. The application of Chlorpropham (CIPC) sprout suppressant, as a thermal fog is associated with a deterioration in fry colour. The BPC funded project at the University of Glasgow and its collaborator Sutton Bridge Experimental Unit investigates the effects of CIPC use on the processing quality of stored potatoes.

CIPC is the only sprout suppressant available for medium and long-term storage for processing in Britain. In the UK the majority of CIPC treatments are conducted as thermal fog applications. This is considered to be the most practical means of achieving successful sprout control.

The introduction of a hot fog into potato stores has a disruptive influence. It can physiologically alter the potatoes by creating a stressful environment. Tuber respiration rate increases and so the crop will age. Experimental trials conducted as part of this project have shown that it is the fogging process itself that is responsible for the decrease in crop quality following application, not the CIPC formulation applied.

Studies revealed that both carbon dioxide and ethylene were produced naturally by crop and from the combustion of petrol used to generate thermal fogs. Initially the fry colour problems were linked with carbon dioxide in combustion gases and from increased respiration. However carbon dioxide output from thermal fogger machines was less significant than expected. The levels were consistently lower than concentrations shown to have a deleterious effect in previous BPC funded work.

Ethylene is present in thermal fogs as a by-product of burning the hydrocarbon fuel used to generate the fog. The concentration of ethylene produced is associated with the running conditions of the fogger machine I.e. burner temperature, type and volume of fuel used etc. The ethylene created in a standard CIPC thermal-fog application is sufficient to induce a physiological response in tubers. Exposure of crop to ethylene effects respiration, dormancy period, sprout morphology, reducing sugar concentration and hence fry colour. The extent of the outcome depends on exposure time and concentration.

Following assessment of the fogging situation, various means of reducing the impact of CIPC application on fry colour were evaluated. Different approaches were undertaken and included both attempting to control and remove the contaminants present in thermal fogs.

By ventilating stores earlier than the recommended twenty-four hour period after treatment a vast improvement in fry colour was observed. In doing this the exposure time of crop to contaminants was greatly reduced. In the experimental work the stores were ventilated eight hours after treatment. This allowed adequate time for the effective fraction of the thermal fog to settle. Currently ventilating stores earlier then the stated twenty-four hour period is not in accordance with the formulation labels' recommended procedure. However data from this project is being used to aide the case for changes to label recommendations put to formulation manufacturers.

Generating less contamination when fogging was a further successful strategy for minimising the impact on fry colour. To do this 'cleaner' simpler fuels were used to create the thermal fogs. A clear improvement was seen when the CIPC treatment was carried out using methanol fuel. This bio-fuel is renewable however is considerably more expensive than petrol (the current standard) when fuel consumption and cost per unit volume are considered. LPG (liquefied petroleum gas) was another fuel studied. The distinction in quality between crop treated using petrol fuel and that treated with LPG was marginal. Improved fry colours were expected using LPG. This outcome was a consequence of an inefficient burner system in the fogger machine that had been adapted to burn LPG. The resulting fry colours are related to the excess of fuel consumed than would be the case if the burner had been fully optimised. The LPG system is continuingly being manipulated to obtain maximum efficiency, and is very much an option worth developing. Compared with petrol, LPG is cheap and clean, and is likely to offer improved fry colours and improved economy.

The impact of repeated fog applications throughout a season was investigated. By reducing the total number of fog applications fry colour was allowed to recover to a greater extent and was more predictable over the course of the storage period. By altering the rate of CIPC application, ensuring the same overall tailored dose was delivered in a season, the total number of treatments required was cut down. Less fogging means less frequent fog contamination in store. This work is experimental and is not covered by current label recommendations. The methodology has to be tested on a wider scale but it is a promising move toward resolving fry colour concerns.

Preliminary trials looking at reducing/removing fog contamination in stores have indicated the following. For an absorbent/scrubbing system to be physically and economically effective it would have to (i) be dynamic (forced air exchange with the store atmosphere) (ii) employ an affordable material with a high affinity for a specific range of compounds. Those materials tested so far have shown some improvement in crop quality. For optimum results the absorbent had to be regenerated frequently, introducing an additional complication. Continued research would be beneficial in this area.

The best improvements in fry colour post-fogging would be achieved by avoiding hydrocarbon fuels to generate thermal fogs. There are machines in the developmental stages that will do this, however the integration of such equipment into routine commercial procedure is still some way off. Therefore in the prevailing period modifications to fogging practices such as those discussed are the best means of minimizing the detrimental impact that CIPC use has the processing quality of stored potatoes.

List of Contents

Abstract i
List of Contents iv
List of Tables vi
List of Figures viii
Dedication xi
Acknowledgements xii
Declaration xiii

1 **Chapter One** 1
1.1 INTRODUCTION 1
1.1.1 Background to the potato industry in the context of long-term storage 1
1.1.2 Changes in the potato during storage 4
1.1.3 Methods of sprout control 9
Chlorpropham 12
1.1.5 Colour development on processing 18
1.2 Aims 21

2 **Chapter Two** 23
2.1 MATERIALS AND METHODS 23
2.1.1 Ethylene determination 23
2.1.2 Analysis of carbon dioxide gas 30
2.1.3 Fry colour assessment as an indicator of potato processing quality 32
2.1.4 Sugar analysis 41
2.1.5 Auxiliary materials and methods used throughout the project 46
2.1.6 Statistical Analysis 49

3 **Chapter Three** 52
3.1 CORRELATIONS FOR METHOD VALIDATION 52
3.1.1 Systems of detection used for ethylene analysis: Gastec compared with Gas Chromatography 52
3.1.2 Methods of assessing processing quality: Reducing sugar concentration compared with fry colour examination 62
3.1.3 Analysis methods of reducing sugar concentration: Roe, Leloir-Roe and Somogyi-Nelson methods compared with High Performance Liquid Chromatography method 75

4 **Chapter Four** 76
4.1 ASSESSMENTS OF THERMAL FOGGING MACHINES 76
4.1.1 The thermal fogging process 76
4.1.2 Assessment of the gases produced by thermal fogging machines in a commercial store 82
4.1.3 Assessment to determine wether Ethylene is produced by the thermal fogging process 84
4.1.4 Assessment of representative commercial thermal fogging machines 85
4.1.5 Secondary trials with representative commercial thermal fogging machines 89
4.1.6 The effect of thermal fog application on the processing quality of stored potatoes 92

4.1.7 Features of the thermal fog application process that can potentially alter the quality of stored potatoes 99
4.1.8 The Combustion process 103
4.1.9 Fuel consumption for lead replacement petrol 107
4.1.10 Alternative fuels 108
4.1.11 Predicted levels of ethylene introduced to store atmospheres by thermal fog application 110
4.1.12 The energy created by a thermal fog application 111
4.1.13 Conclusions 114

5 **Chapter Five** 116
5.1 IDENTIFYING WHEN PROBLEMS OCCUR AND VERIFYING THAT ETHYLENE HAS AN EFFECT ON PROCESSING QUALITY 116
5.1.1 Background Information on Ethylene 116
5.1.2 Natural levels of ethylene production by potatoes in storage 121
5.1.3 Crop storage trial to determine the effect of ethylene on processing quality 126
5.1.4 Potato store atmosphere samples 132
5.1.5 Persistence of ethylene in the store atmosphere 136
5.1.6 The effect of exposure to ethylene with varying concentration and time ..147
5.1.7 Conclusions 162

6 **Chapter Six** 163
6.1 CONTINUOUS EXPOSURE OF POTATOES TO AN ETHYLENE ATMOSPHERE 163
6.1.1 Introduction 163
6.1.2 Continuous exposure experimental work 168
6.1.3 Commercial store using ethylene as a sprout suppressant 191
6.1.4 Conclusions 194

7 **Chapter Seven** 195
7.1 POTENTIAL METHODS CONSIDERED FOR REDUCING THE IMPACT OF THERMAL FOG APPLICATION ON PROCESSING QUALITY 195
7.1.1 Introduction 195
7.1.2 Investigation of methods of control 198
7.1.3 Investigation of methods of removal 226
7.1.4 Conclusions 241

8 **Chapter Eight** 243
8.1 FURTHER RELEVANT INVESTIGATIONS INTO POTATO CHARACTERISTICS AND BEHAVIOUR 243
8.1.1 Potato volatiles 244
8.1.2 GCMS work on volatiles 246
8.1.3 Crop condition trial 252

9 **Chapter Nine** 261
9.1 Conclusions 261
9.1.1 Outcomes this project 261
9.1.2 Update on the situation in the potato industry 264

10 **References** 267

List of Tables

Table 1 Examples of government CIPC residue levels found on potato samples collected from various retail outlets in 2000. 16
Table 2 Example of statistical analysis (Day 14 results from correlation trial (p64) 50
Table 3 Concentrations of ethylene spikes used in correlating methods of determination..55
Table 4 Determination of ethylene concentration by GC and Gastec (vessel 1) 55
Table 5 Observations on the Gastec readings from Vessel 1 56
Table 6 Determination of ethylene concentration by GC and Gastec (vessel 2) 58
Table 7 Observations on the gastec readings from vessel 2 59
Table 8 Statistical analysis of L-values. Different letters denote significant differences...64
Table 9 Statistical analysis of glucose levels. Different letters denote significant differences 68
Table 10 Statistical analysis of fructose levels. Different letters denote significant differences 68
Table 11 Design of fogger machine assessment trial 85
Table 12 Details of the treatments conducted for assessment of representative thermal fogging machines (application temperatures in degrees Celsius) 86
Table 13 Ethylene concentrations in store atmospheres after thermal fog application (burner temperatures in degrees celcius) 87
Table 14 Details of treatments conducted for trials to verify ethylene is produced by the thermal fogging application process 89
Table 15 Carbon dioxide concentrations in store atmospheres over a two-week period following fogging 90
Table 16 Ethylene concentration in store atmospheres following fogging 91
Table 17 Treatments conducted to determine the effect of thermal fog application on processing quality 92
Table 18 Carbon dioxide concentration in store atmospheres following treatment 93
Table 19 Ethylene concentration in store atmospheres following fogging 93
Table 20 Statistical analysis of L-values. Different letters denote significant differences.94
Table 21 Treatments conducted to assess which stage(s) of the application process has the detrimental impact on processing quality. 96
Table 22 Statistical analysis of L-values. Different letters denote significant differences.98
Table 23 Volume of petrol consumed in a fifteen-minute period (ml) 107
Table 24 The volume of methanol consumed in a fifteen-minute period (ml) 109
Table 25 Weight of LPG (propane) consumed in a fifteen-minute period (g) 109
Table 26 The energy produced by the combustion of petrol (based on an average value of petrol consumption at each burner temperature) 111
Table 27 The volume of fuel required to run a thermal fogging machine at the specified burner temperature for a fifteen-minute period 113
Table 28 Comparison of methanol volumes measured and theoretical for fog generation. 113
Table 29 Summary of environmental conditions used in treatments to study natural ethylene production 122
Table 30 Tubers in normal storage compared with elevated CO_2 storage conditions 124
Table 31 Tubers in normal storage compared with elevated temperature conditions 124
Table 32 Tubers in normal storage compared with an external source of ethylene 124
Table 33 The treatments compared for effect of ethylene on processing quality 128
Table 34 Statistical analysis of L-values. Different letters denote significant differences 129
Table 35 Mean ethylene concentration in store atmospheres over time resulting from individual treatments 131

Table 36 Ethylene concentration in 5l store atmosphere samples collected following fogging 135
Table 37 Treatments conducted in trial investigating the persistence of ethylene in store atmospheres. 139
Table 38 Treatments conducted in trial investigating the effect of exposure of ethylene with varying concentration and time. 149
Table 39 Statistical analysis of L-values after 8 hour treatment. Different letters denote significant differences 151
Table 40 Statistical analysis of L-values after 24 hour treatment. Different letters denote significant differences 151
Figure 41 The effect of exposure to ethylene on fry colour-DAY 1 152
Figure 42 The effect of exposure to ethylene on fry colour-DAY 7 153
Table 43 Statistical analysis of Sprouting data. Different letters denote significant differences 161
Table 44 Summary of treatments involving ethylene exposure using apples as a source .170
Table 45 Headspace volume remaining in the bottles after both solutions are added 178
Table 46 Ethylene concentration in headspace generated by different Ethrel to saturated sodium carbonate ratios 179
Table 47 Release rates of ethylene from the different solution ratios over time 179
Table 48 The ratio of Ethrel to releasing agent for a range of ethylene concentrations (set 1) 181
Table 49 The ratio of Ethrel to releasing agent for a range of ethylene concentrations (set 2) 181
Table 50 The total volume of ethylene present in the headspace of boxes 184
Table 51 Statistical analysis of glucose levels. Different letters denote significant differences 190
Table 52 Statistical analysis of fructose levels. Different letters denote significant differences 190
Table 53 Ethylene concentration in tin sample of commercial store atmosphere 193
Table 54 Statistical analysis of L values –May 01. Different letters denote significant differences 200
Table 55 Statistical analysis of L-values-November 2001. Different letters denote significant differences 203
Table 56 Statistical analysis of defects-November 2001. Different letters denote significant differences 204
Table 57 Statistical analysis of L-values-May 01. Different letters denote significant differences 210
Table 58 Statistical analysis of L-values-Nov 01. Different letters denote significant differences 214
Table 59 Statistical analysis of defects-November 01. Different letters denote significant differences 214
Table 60 Summary of fuel properties 216
Table 61 Statistical analysis of L-values. Different letters denote significant differences 220
Table 62 Statistical analysis of defects. Different letters denote significant differences..221
Table 63 Percentage loss of ethylene from the storage containers over time 233
Table 64 Respiration rate of samples in storage containers over time 234
Table 65 List of volatiles of interest evolved by potatoes in storage 247
Table 66 Measurements of air speed exiting hair dryer unit (m/sec) 254
Table 67 Summary of treatments and application carried out in Crop condition trial 255
Table 68 Mean ethylene concentration in the headspace of boxes receiving forced air treatment (ppb) 257
Table 69 Mean respiration rate of potatoes exposed to the stress of hot or cold air at force for one or two minutes 259

List of Figures

Figure 1 Structure of CIPC 12
Figure 2 Cross-section of the potato tuber showing internal structure (*Woolfe, 1987*) 13
Figure 3 Three-dimensional colour coordinate system 38
Figure 4 Gastec colorimetric detector tube for ethylene analysis in the concentration range 0.2-50ppm 52
Figure 5 5l dilution vessel used for determining the concentration of ethylene using both methods 54
Figure 6 Scale divisions on a Gastec tube as used in the experiment 56
Figure 7 Graph of the relationship between measured and actual ethylene concentrations using two methods of determination (vessel 1) 57
Figure 8 Graph of the correlation between ethylene determination by two methods (vessel 1) 58
Figure 9 Graph of the relationship between measured and actual ethylene concentrations using two methods of analysis (vessel 2) 59
Figure 10 Graph of the correlation between ethylene determination by two methods (vessel 2) 60
Figure 11 The effect of storage temperature on the fry colour of potatoes (n=3) 64
Figure 12 The effect of low temperature storage on reducing sugar levels over a short period 66
Figure 13 The effect of warm temperature storage on reducing sugar levels over a short time 67
Figure 14 The effect of optimum storage temperature on reducing sugar levels over a short time 68
Figure 15 Graph of the relationship between reducing sugar content and fry colour 70
Figure 16 Graph of the relationship between fry colour and fructose content 71
Figure 17 Graph of the relationship between fry colour and glucose content 71
Figure 18 Fry colour chart for crisping potatoes (*BPC, technology transfer*). 74
Figure 19 Diagram of the Swingfog machine formerly used in common practice for CIPC application in stores 76
Figure 20 Schematic diagram of the working principles of the Swingfog machine 77
Figure 21 Representative commercial fogger machine in use currently in the UK (SCC, Unifog) 77
Figure 22 Schematic diagram of the thermal fogging process 79
Figure 23 Control panel on commercial fogging machine used for controlling application conditions 81
Figure 24 Layout of the M^{C}Cain stores at Cayton, Scarborough 82
Figure 25 The effect of thermal fogging on fry colour during storage (n=4) 94
Figure 26 The effect of the thermal fogging process on the aesthetic quality of crisps 95
Figure 27 The effect of the fogging process on fry colour during storage (n=2) 97
Figure 28 Interactions between the plant and its environment 117
Figure 29 The structure of ethylene 118
Figure 30 Schematic diagram of a potato tuber cut through its transverse axis. Not to scale. (*MacKinnon, 2000*) 119
Figure 31 Comparison of the effect of ethylene from different sources on fry colour (n=3) 129
Figure 32 Comparison of the effect of ethylene from different sources on % fry defects. 130
Figure 33 Schematic diagram of 5l tins designed for collecting and transporting store atmosphere samples 133
Figure 34 Mean % of applied ethylene present in atmosphere over an 8 hour period 140
Figure 35 Mean % of applied ethylene present over 8 hours including standard deviations (n=3) 141

Figure 36 Comparison of the persistence of ethylene over 8 hours with and without the presence of tubers....142
Figure 37 Mean % of applied ethylene present in atmosphere over a 24 hour period....143
Figure 38 Mean % of ethylene applied present over 24 hours including standard deviations (n=3)....144
Figure 39 Comparison of the persistence of ethylene over 24 hours with and without the presence of tubers....145
Figure 40 Comparison of the effect on fry colour by different concentrations and exposure times of potatoes to ethylene (n=3)....150
Figure 41 The effect of exposure to ethylene on fry colour-DAY 1....152
Figure 42 The effect of exposure to ethylene on fry colour-DAY 7....153
Figure 43 The effect of exposure to ethylene on fry colour-DAY 14....154
Figure 44 The effect of exposure to ethylene on fry colour-DAY-28....154
Figure 45 Comparison of the effect on potato respiration rates by exposure to ethylene for 8 hours at different concentrations (n=3)....156
Figure 46 Comparison of the effect on potato respiration rates by exposure to ethylene for 24 hours at different concentrations (n=3)....158
Figure 47 Mean length of the longest sprout-8hour exposure time (n=3)....160
Figure 48 Mean length of the longest sprout 24hour exposure (n=3)....160
Figure 49 Tubers from GU trials in which they were exposed to an external source of ethylene....164
Figure 50 The mean atmospheric ethylene concentration in boxes resulting from treatment....172
Figure 51 Mean respiration rates on day 28 resulting from treatments....174
Figure 52 Sprouting on tubers that were not exposed to ethylene for the duration of the trial....175
Figure 53 Sprouting on tubers that were exposed to ethylene for the first seven days of the storage term....175
Figure 54 Sprouting on tubers that were exposed to ethylene for the first 21 days of the storage term....176
Figure 55 Sprouting on tubers that were exposed to ethylene for the entire duration of the storage term (28 days)....176
Figure 56 The mean ethylene concentration in headspace of boxes resulting from individual treatments....185
Figure 57 Mean reducing sugar concentration resulting from storage conditions....186
Figure 58 Mean reducing sugar concentration of samples exposed to ethylene in storage (low)....187
Figure 59 Mean reducing sugar concentration of samples exposed to ethylene in storage (medium)....188
Figure 60 Mean reducing sugar concentration of samples exposed to ethylene in storage (High)....189
Figure 61 The effect of ventilation time on fry colour (n=4, May 2001)....200
Figure 62 The effect of ventilation time on fry defects (n=4, May 2001)....200
Figure 63 The effect of ventilation time on the fry quality of crisps. Representative samples from each treatment over the storage time....201
Figure 64 The effect of ventilation time on fry colour (n=8, November 2001)....203
Figure 65 The effect of ventilation time of fry defects (n=8, November 2001)....203
Figure 66 The effect of fuel type used in fogging on fry colour (n=4, May 2001)....210
Figure 67 The effect of fuel type used in fogging on fry defects (n=4, May 2001)....211
Figure 68 The effect of alternative fuel use on the aesthetic quality of crisps. Representative samples from each treatment over the storage term (May 2001)....212
Figure 69 The effect of fuel type used in fogging on fry colour (n=8, November 2001)..213
Figure 70 The effect of fuel type used for fogging on fry defects (n=8, November 2001)....214

Figure 71 The effect of dose rate per application on fry colour over time (n=4) 220
Figure 72 The effect of dose rate per application on fry defects over time (n=4) 221
Figure 73 The pattern of fry colour results from four applications at half dose 222
Figure 74 The pattern of fry colour results from two applications at full dose 222
Figure 75 The pattern of fry colour results from one application at double dose.....223
Figure 76 The difference in fry colour (L-units) between sampling occasions 224
Figure 77 Reducing sugar concentration in the control samples over time (2l) 230
Figure 78 Reducing sugar concentration when ethylene is removed from the atmosphere (2l) 231
Figure 79 Reducing sugar concentration when an elevated concentration of carbon dioxide is created in the atmosphere (2l) 231
Figure 80 Reducing sugar concentration when both ethylene and carbon dioxide are removed from the atmosphere (2l) 232
Figure 81 Reducing sugar concentration in the control samples over time (5l) 235
Figure 82 Reducing sugar concentration when ethylene is removed from the atmosphere (5l) 236
Figure 83 Reducing sugar concentration when carbon dioxide levels are elevated in the atmosphere (5l).....237
Figure 84 Reducing sugar concentration when ethylene and carbon dioxide was removed from the atmosphere (5l).....238

Dedication

To Mum, you taught me to keep my aspirations in the sky and my feet on the ground. Thank you

Acknowledgements

Firstly I have to thank Dr Harry Duncan for his patience and confidence in me. Harry's ability to make me think that little bit more each and every time has made all the difference. It's much appreciated.

Michael Beglan you are a god send! Without your experience, help and advice this thesis would not be possible. Many thanks.

Thanks to my friend, Ms Laura Park for numerous mutual venting of frustrations and the kind of understanding you only get from another potato PhD student.

I'm grateful to everyone in EAA chemistry for providing a good working environment, coffee and nice things to eat.

I'd like to express my gratitude for all the assistance and good humour from the staff at Sutton Bridge Experimental Unit, particularly Adrian Briddon, Adrian Cunnington, Ajay Jina and Geoff Wright.

I appreciate all commercial help with providing samples (M^{c}Cains, Greenvale and MBM), analysis for correlation purposes (Walkers) and countless thermal fog applications (Stored Crop Ltd, Superfog Ltd, Sands Agricultural Machinery and Luxan). I am indebted to Nick Green, Tom Green and Pat Nix of Stored Crop Ltd whose input has always been positive, flexible and enthusiastic.

Recognition goes to the British Potato Council and the Potato Processors Association for the funding of this project. Thanks to Dr Mike Kirkman for his editorial input and support.

Special thanks to my parents, brothers and sisters for their continued support and encouragement. Thanks to John-Ross and Airyn for the Missus Potato Head toy and many, many laughs and smiles that help to keep things in perspective.

Finally, I acknowledge whole-heartedly the unreserved strength, energy, love and understanding of my partner Michael. Thank you for putting up with me.

Declaration

Except where specific reference is made to other sources, the work presented here is the original work of the author. It has not been submitted, in part or in whole, for any other degree. Some of the results have been presented elsewhere.

Geraldine Dowd

1 Chapter One

1.1 INTRODUCTION

1.1.1 Background to the potato industry in the context of long-term storage

This project was started in late 1999 and the information presented here is the background to the situation in the potato industry as it was then. Any advancements or changes to recognised procedures since this time have been addressed throughout the thesis as they became relevant.

The potato is ranked fourth in the world in production of all agricultural commodities. The expansion of the processing sector in countries with developed market economies substantiates that consumer preferences for crisps and French fries is the driving force behind this (*Duplessis et al, 1996*). In the UK this trend is also evident, with a big increase in the tonnage used for both table potatoes and processed goods, although the increase in the processing market was comparatively smaller. Between 1990 and 2000 there was a 35% increase in processing tonnage and a 62% increase in frozen and chilled sector tonnage (*R.. Harris, personal communication*). Recent figures show that circa 4.7 million tonnes of ware potatoes are stored in the UK on a yearly basis. It is estimated that the retail value of the processing sector is approximately £3.7 billion in the UK (*R. Harris, personal communication*).

Not only does the potato industry as a whole require a year round supply of crop, but consumers also expect this continued availability of good quality produce regardless of season. *Suttle, 1996,* stated that in many low-income households potatoes are the principal source of dietary fibre. The UK climate means that there is a defined growing season for potatoes in this country and continuous growing is not possible. Therefore potatoes have to be stored after harvest or imported from countries with different growing seasons (*Duncan et al, 1992*). The notion of seasonal foodstuffs is all but lost in society today. Notwithstanding the mounting pressure from groups opting for 'organic' diets and the implied seasonal nature of this as a long-term option, seasonality is not a consideration for the majority. The few public spheres in which it remains prevalent tend to be at either ends of the economic scale i.e. expensive restaurants and home grown agricultural goods. In the UK and in many other countries year round availability of both table potatoes and

processed products is expected and relied upon as a stable base in most weekly shopping baskets. To meet this requirement long-term storage of harvested crop is essential.

Following harvest potatoes have a period of time during which they are dormant and sprout growth will not occur. In general terms dormancy is broken when sprout growth begins (*Coleman, 1987, Suttle, 1996, Wiltshire & Cobb, 1996*). The timing of initiation and the ensuing rate of growth can be slowed markedly by storing at temperatures of 5°C or less. Processing quality will be forfeited if this method is used because low temperatures give way to starch conversion and correspondingly reducing sugar accumulation. Therefore storage at a higher temperature is used for potatoes intended for the processing market. Unfortunately at these temperatures tubers will sprout.

Providers of ware potatoes and in particular the processing sector depend on the use of the sprout suppressant herbicide CIPC (isopropyl 3-chlorophenylcarbamate), also known as Chlorpropham, to enable potatoes to maintain water, energy and weight and thus high quality while in storage for periods of up to nine months. The key features of potato quality are the appearance in terms of colour, the taste, and the texture. The most influential quality parameter for processing potatoes is reducing sugar concentration, which ultimately decides the value of the crop. It directly affects the lightness and taste of the processed product. Any disease or rot of the crop obviously reduces market acceptability also. Pre-pack or table potatoes do not have such stringent requirements in terms of sugar content. Providing the tubers have a good skin finish and no blemishes or greening they are generally acceptable for sale. Conventionally CIPC was only used on crop intended for processing. However with increasing pressure on the pre-pack market to provide a wider variety of cultivars and an extended shelf life CIPC use has become commonplace.

The measurement of reducing sugars consists of the glucose content plus the fructose content. The measurement of total sugars refers to this value plus sucrose. These two reducing sugars are produced on hydrolysis of sucrose.

The rising public concerns over food safety have brought about demands from potato product manufacturers and supermarkets that very little or no pesticides be used on source crop. This general opinion seems to be based on limited insight of what these specific pesticides are and what function they provide. Overall the consensus among consumers is that all pesticides have a negative impact on your health. This perception clearly has strong foundations but would benefit from further basic knowledge about individual

chemicals or groups of them. It is apparent that although the public have misgivings over pesticide residues the majority of people are not willing to forego the ease of availability of 'fresh' produce or the snack orientated lifestyle that is so prevalent nowadays. The public attitude is exemplified through the demands made by multiple retailers in that although they are aiming for low or no residues, by restricting the use of chemicals they continue to expect the same high quality produce after months of storage. This is only a brief description of the problems faced by the potato industry regarding provision of acceptable potatoes. In essence a balance has to be struck between optimised use of pesticides for maximum benefit and maintaining the provision of safe and healthy potatoes for all purposes.

The majority of facilities in the UK tend to use tonne boxes for storage rather than bulk. Commonly stores have high capacities of one or two thousands of tonnes rather than 500 hundred tonnes or smaller. However some of these smaller stores remain and house the diminishing number of small-scale growers and producers. Box stores are easier to manage than bulk. They have better airflow through and around them and allow easier monitoring for disease and sprouting. Quality assurance schemes are considerably easier to put in place as each consignment of boxes can be labelled and traced from arrival to departure. In bulk stores the opportunity for selection and removal of crop from store at intermittent stages of storage is much more limited.

.

1.1.2 Changes in the potato during storage

Starch constitutes around 65-75% of potato tuber dry matter (*Boyd, 1988, Burton, 1989*). The total amount of dry matter depends on the amount of water lost by evaporation and transpiration. Respiration even under settled conditions decreases the starch supply. The mechanism of starch degradation, leading to the amassing of reducing sugars, occurs mainly via the action of phosphorylase and invertase reactions (*Mares et al, 1985*).

Senescent sweetening is the irreversible conversion of starch to sugars that occurs when potatoes are stored for long periods of time, usually at temperatures of 8-12°C. Although in most situations the major products are glucose and fructose and in some instances sucrose is also accumulated. This means that normally after crop has been in storage for a long period it will have an increased level of reducing sugars, a darker fry colour and be worth nothing in the market. The sweetening occurs as the tubers physiologically age and lose weight through respiration and sprout growth.

The process of starch conversion is generally accepted as the reason for poorer quality following extended storage. An alternative view suggested by *Brierley et al, 1997* is that the complex nitrogen flux during prolonged storage might be the cause. Protein breakdown occurs during late storage causing an accumulation of free amino acids:

> "The increase of these amides upon emergence from dormancy, may account for the decline in potato tuber processing quality often observed after prolonged storage"

In some cases this build-up corresponded with a deterioration in processing quality that was not reflected by reducing sugar content (*Brierley et al, 1996*). The researchers postulated that amino acids had a synergistic effect on fry colour and late storage resulted in darker fry colour per unit of reducing sugar than in earlier storage.

Regardless of whether amino acid content influences the extent of darkening it is normal for processing quality to decline over the course of the storage season. Therefore it is common for crisps to have a darker fry colour toward the end of the season, from April/May onwards. The differences can seem quite extreme when compared to fry colours of crisps from November the previous year.

A recent study (*Copp et al, 2000*) has shown a trend between the timing of an increase of respiration rate during storage and the onset of the decline in chip colour quality,

suggesting that measurements of respiration rate could potentially be used as a non-destructive in situ method of predicting the onset of senescence.

1.1.2.1 Factors that influence Reducing sugar levels during storage

Although the pattern of sugar behaviour varies between cultivars it can be affected by pre-harvest factors such as damage at harvest, fertilizer type and quantity, moisture stress, soil temperature and latitude of production. Sprouting patterns can also be influenced by features such cultivar and season (*Hay & Hampson, 1991*). Once harvested the main factors that influence the dynamic equilibrium between starch and glucose, fructose and sucrose are outlined in the following pages.

1.1.2.2 Harvest time

The more mature a crop is when it is lifted the less sucrose is present. By allowing potatoes to mature to a reasonable stage before lifting the amount of sucrose available to then be converted into free reducing sugars is less and thus any subsequent decline in processing quality will be limited (*Sowokinos, 1973*).

Hertog et al, 1997 found that:

> "The maturity at the time of harvest determines the storage behaviour through the initial amount of the enzyme (or enzyme system) responsible for cold induced sweetening."

Therefore the potential for cold induced sweetening and sugar stability in general depends upon the physical and chemical maturity of the crop when it enters the store.

1.1.2.3 Preconditioning

If a crop is chemically immature after harvest it can be preconditioned by storage at an elevated temperature of around 15°C for a number of weeks, usually four or five. This instigates a decline in sugar content. After preconditioning the temperature is gradually lowered to the optimum for general storage.

1.1.2.4 Curing/would healing

It is normal for potatoes not to have fully developed skin after harvest and perhaps suffer slight surface damage during store loading. A curing period allows the skin to fully

develop/heal in a short time because of the higher temperature and lower relative humidity conditions than used for general storage. Biologically the process is termed suberization because it is the deposition of suberin (a fatty substance in the cork tissue used for protection from disease). There are two main stages of deposition in the process-firstly the polyphenolic compounds followed by polyaliphatic compounds (*Suttle & Lulai, www.npcsspud.com/newpage118.htm, February 2000*). Temperatures of 15-18°C for a duration of approximately two weeks are allowed for curing to be completed. The same effect is observed as with preconditioning in that sugar levels decline.

1.1.2.5 Low temperature storage

It is well documented that exposure of potatoes to low temperatures of approximately 2 - 5°C induces the breakdown of starch and correspondingly the accumulation of sucrose, glucose and fructose (*Barichello et al, 1991, Marangoni et al, 1996, Marangoni et al, 1997, Espen et al, 1999*). This process is known as low temperature sweetening and causes darker fry colour and an associated bitter taste (*Uppal, 1999*). The exact mechanism through which it occurs has yet to be fully described. It is known that exposure to low temperatures affects various metabolic pathways (*Nowak, 1977*). A lot of the research on this subject has focused on the starch properties and to what extent they are responsible for starch conversion at low temperatures. The ratio of amylose to amylopection appears to be critical in determining the cultivar sensitivity to low temperature sweetening. The more amylose that is present the more ordered and densely packed the crystalline regions are, which makes the starch grains more stable. Greater stability equals greater resistance (*Barichello et al, 1991*).

If storage at low temperatures is temporary then reconditioning can reverse the damage, but long-term storage in the cold leads to irreversible sweetening.

1.1.2.6 General storage conditions

Aside from temperature other storage parameters can affect the reducing sugar accumulation such as duration of storage, sprout suppressants applied, relative humidity, ventilation and the use of controlled atmospheres. In particular control of relative humidity and storage temperature is imperative as they impact on the extent of water loss and as such weight loss (*Cargill et al, 1971*). This in turn influences the dry matter content (*Laza et al, 2001*).

Yang et al, 1999, found that treatment with CIPC can affect sugar levels and the free amino acid content of potatoes, but after sixteen weeks of storage the fry colour was acceptable for processing. Earlier work by *Nowak, 1977*, showed that CIPC treatment prevented changes in the soluble protein fractions.

Storage in bulk rather than boxes can have a stressful impact on potatoes leading to enhanced reducing sugar levels. Tubers are most vulnerable to pressure from static loading in bulk storage (*Hironaka et al, 2001).* Additionally bulk storage can lead to the accumulation of potentially detrimental volatile compounds within the pile because of the lack of air movement through the stack.

Carbon dioxide levels have to be monitored to avoid a build up in store, which would increase reducing sugars. To achieve this adequate ventilation has to be maintained (*Peet,* www.cals.ncsu.edu/sustainable/peet/profiles/harv_pot.html, *February, 2000*).

In general terms other considerations for maintaining quality during storage include avoiding light in stores whenever possible to prevent chlorophyll and glycoalkaloid formation, and ensuring there is adequate insulation for better control of temperature (*www.css.orst.edu/potatoes/storproc.htm, February 2000*).

1.1.2.7 Reconditioning

Reconditioning is the process of raising the storage temperature to cause some of the sugars to be re-synthesised into starch thereby decreasing the reducing sugar content. Storage temperature significantly affects the ability of potatoes to recondition to an adequate extent (*Cargill et al, 1971, Peshin, 2000*). The higher temperature also causes increased respiration which itself consumes a small proportion of the reducing sugars. This approach is adopted mostly after low temperature sweetening has occurred in an attempt to improve quality.

The storage temperature is increased to between 15-20°C for a period of approximately three to four weeks before leaving the store and going to the processor (*Pritchard & Adam, 1994*).

Bearing in mind the potential influence that amino acid content can have on fry colour, it is important to note that reconditioning is ineffective as a means of decreasing the free amino acid content (*Brierley et al 1996*).

1.1.2.8 Handling stress

Handling of potatoes, both pre and post-storage causes stress. This is usually manifested physiologically by an increase in respiration and reducing sugar development. Undue stress from handling is avoided as far as possible, but simple processes such as loading and unloading stores takes it toll on the physical state of the potatoes. Any increase in respiration and sugar conversion is undesirable and a step towards dormancy break.

The response to handling is cultivar dependant. *Orr et al, 1994,* studied a potential means of predicting responses of potatoes to handling in an attempt to avoid poor quality processed foods. They concluded that a handling simulator could be used to standardize the response of a cultivar to industrial handling and potentially could be integrated into breeding programs that would allow this prediction for new cultivars.

What now follows is a resume of the sprout suppressant chemical situation that existed in late 1999 when this work began. Any modifications in permitted use will be addressed at some point throughout the project.

1.1.3 Methods of sprout control

1.1.3.1 Maleic Hydrazide (MH)

This is a foliage-applied treatment that penetrates the cuticles of the leaves and is translocated through the phloem to the tubers. Many factors can influence the uptake of the chemical and hence its success in controlling sprouting. These include timing of application (optimised leaf penetration), weather conditions, distribution of chemical between tubers. It is considered that a dose of 10mg/kg is sufficient to control sprouting. Any level higher than this may raise safety concerns because the chemical is present inside the flesh of the potato as opposed to on the skin, like most other post-harvest treatments for sprout control (*Duncan et al, 1992*). Presently MH does not tend to be used alone for sprout control, but rather within a regime involving post harvest chemicals. It is generally perceived that treatment in the field with MH can increase the time before the first post harvest treatment of a sprout suppressant is required.

An additional beneficial use of MH that is claimed is for groundkeeper control to prevent volunteer infestation of the following crop.

1.1.3.2 Controlled Atmosphere Storage (CAS)

Although controlled atmosphere storage has been shown to be effective for many fruits and vegetables (*Kader, 1986, Herregods, 1995, Mathooko, 1996*) it has had limited success in the potato storage business for a number of reasons. It involves altering the gas balance within storage facilities to manipulate conditions that will slow down respiration and hence growth of sprouts by extending dormancy. Most often the oxygen levels are reduced to much less than that of atmospheric levels, from ~21% down to as low at ~3%. This is usually done in conjunction with increasing the level of carbon dioxide from less than 1% up to around 5 or even near 10% (*Khanbari & Thompson, 1996*). The exact mechanism by which enhanced carbon dioxide regulates respiratory metabolism is unclear (*Mathooko, 1996*). It is well known that increased levels of carbon dioxide in stores leads to a decline in quality and consequently a darker fry colour (*Mazza & Siemens, 1990, Briddon & Jina, 1997*). Prolonged storage of potatoes at low oxygen and elevated carbon dioxide levels can also lead to tuber breakdown (*Coleman & M'Inerney 1997*). Controlled atmosphere situations are predominantly used in low temperature storage (and in particular for seed potatoes), which itself is not conducive to good processing quality. The system is costly

(*Emond et al, 1998*) and impacts on quality parameters, therefore does not lend itself well to the processing sector.

Occasionally CAS is used for dormancy release purposes, this usually involves other gases like nitrogen and/or ethylene (*Reust & Gugerli, 1984, Coleman & M'Inerney, 1997, Coleman, 1998*).

1.1.3.3 Tecnazene

Processors use tecnazene as an 'at loading' formulation of dust or granules. This is to control sprouting before the first application of CIPC can be made when wound healing is complete. Tecnazene, an organochlorine, does not interfere with the process. Presently the chemical and its metabolites are under scrutiny and the continued use of this product on food crops is uncertain.

1.1.3.4 Propham

Similar to CIPC this is a fairly volatile crystalline compound (isopropylphenylcarbamate). Where Propham was used it was normally in conjunction with CIPC. Its use is no longer permitted in the European Union.

1.1.3.5 Irradiation

Like CIPC this treatment has to be delayed until after the curing period because it interferes with wound healing. Irradiation treatment is performed in a single dose and is normally irreversible. It does lead to a dose dependant increase in reducing sugars although this is a transitory increase. As a consequence of irradiation treatment potatoes are subject to blackening when they are cooked (*Duncan et al, 1992*). Aside from the public concern over eating irradiated foods, cost, convenience of application (that has to be carried out at an approved site) and the affects on processing quality are enough for processors to avoid using this as a method of sprout control.

1.1.3.6 Dimethylnapthalene (DMN)

This substance is present naturally in potato tubers in very low levels. The most effective isomer in controlling sprouting is 1,4-DMN. The properties of DMN were originally recorded in work done at Glasgow University about twenty years ago (*Beveridge et al, 1983*). This is used in higher concentrations of approximately 20mg/kg on potatoes,

normally within a CIPC regime. DMN is particularly volatile and is applied as a fine mist or vapour. The chemical does not have clearance for use in the UK or the rest of Europe but has been in regular use in the United States for a number of years. There is a case being put forward by the company DormFresh at the moment to introduce DMN as a sprout suppressant treatment in both the UK and continental Europe. Registration in these countries is expected in 2004/2005.

1.1.3.7 Other alternative sprout suppressant treatments

The chemical Carvone, extracted from caraway has been tested for use as a sprout suppressant largely in the Netherlands. Its use has never been particularly widespread in the UK because of volatility and processing quality issues in commercial stores but there is a formulation available on the market. The main selling point for this formulation is that it is extracted from a natural product and therefore is viewed as more acceptable than a synthetic chemical. The cost is relatively high compared to other sprout suppressants.

Another naturally occurring chemical considered for use as a sprout suppressant is ethylene (*Kalt et al, 1999*). It is an endogenous hormone involved in many physiological processes in potatoes and indeed most plant systems. It is an extremely volatile compound that can have a big influence and a wide range of implications. The behaviour and impact of ethylene are considered in great detail throughout the remainder of this thesis and in particular chapters five and six.

Hydrogen peroxide has been investigated for its sprout control properties but thus far no commercial formulation has been shown to be worthwhile in the UK.

The concept of modified cultivars of potatoes that would be resistant to low temperature sweetening is attractive to the potato industry but is unlikely to be taken up on a commercial scale because of the public apprehension and political stance on genetically modified foods.

1.1.4 Chlorpropham

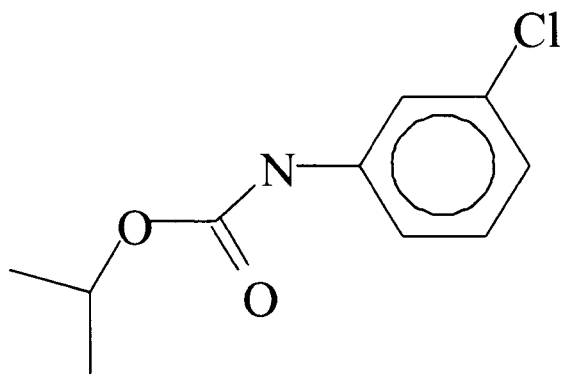


Figure 1 Structure of CIPC

This chemical was first introduced commercially in 1960-1961 in the United States (*Ewing et al, 1968*), but P.C. Martin reported its use as a herbicide in 1952 (Lewis et al, www.kimberly.uidaho.edu/potatoes/cipc.htm). Pittsburgh Plate and Glass Company later patented it. It was registered in the United States in 1962 as a pre and post-emergence herbicide and a plant growth regulator (*Environmental Protection Agency, 1996*). CIPC is a phenylcarbamate and has a wide range of agricultural applications as a herbicide for pre-emergence weed control in grass, blueberries, carrots, onions and garlic to name a few.

It is used to inhibit sprouting in potatoes because it inhibits cell division at the eyes; and is therefore a mitotic inhibitor (*Kirkwood, 1991*). It interferes with organisation of microtubules (*Fedtke, 1982*). The following diagram illustrates the different sections of a potato and clearly shows the positions of the eyes where the CIPC becomes effective. As the eyes open and the cells divide to form sprouts this process is stopped and the tips of the newly developing sprouts become brown in colour. This colour change can indicate clearly where CIPC has been effective and no more sprouting will occur for a further period of storage providing conditions are favourable.

Tubers in this country cannot be exposed to CIPC until after one or two weeks of storage when curing is complete otherwise it would restrict the process and severely damage the crop because it interferes with cell division. Certain parts of Europe can apparently use it 'at loading' and do not have the same level of associated skin or disease problems.

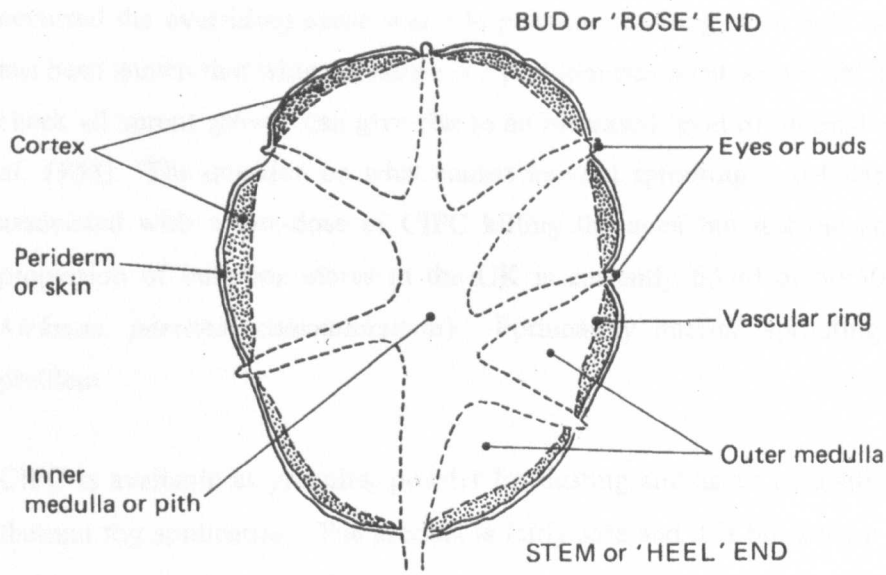


Figure 2 Cross-section of the potato tuber showing internal structure (*Woolfe, 1987*)

Periderm: A protective tissue that has undergone secondary thickening (suberization), consisting of an outer zone of dead cells that is impervious to water and air, with two underlying layers of a corky nature that are slightly more permeable toward the cortex.

Cortex: The layer of plant tissue outside the vascular ring but inside the periderm. It is abundant in storage and phloem cells.

Pith Region: The watery core. The cells in this region are larger and lower in starch content.

Vascular ring: The xylem and inner phloem, whose functions are to transport water and nutrients within the plant, are situated inside the vascular ring. It contains smaller storage cells.

Bud: An undeveloped embryonic shoot containing a meristematic area for cell division. This is where CIPC become effective.

In the past there have been concerns over the involvement of or encouragement by CIPC in developing internal sprouting during storage. In most instances where internal sprouting occurred the overriding cause was pile pressure resulting from bulk storage. However it has been shown that where pressure is a problem treatment with CIPC that does not totally check all sprout growth can give rise to an increased level of internal sprouting (*Ewing et al, 1968*). The question on what causes internal sprouting is debatable but often seems associated with a low-dose of CIPC killing the apex but not the auxiliary buds. The proportion of bulk:box stores in the UK is currently 60:40 or 50:50 for crisps (*Dr M. Kirkman, personal communication*). Fortunately internal sprouting is not a frequent problem.

CIPC is available as granules, powder for dusting and as an emulsifiable concentrate for thermal fog application. The product is fairly safe and it is not known to be carcinogenic, or have any known reproductive, mutagenic or teratogenic effects but can be irritating to skin and eyes and extended inhalation or ingestion may be harmful (www.extoxnet.com; www.nuchem.co.nz, *~late 1999*). In some countries (Denmark, Japan, Germany) there is debate over the safety of CIPC and in Germany at least its use in certain formulations is no longer permitted (*Nagayama & Kikugawa, 1992; Dr H. Duncan, personal communication*). The continued use of CIPC is supported throughout the European agricultural sector.

The environmental fate of CIPC is one of the major concerns surrounding its routine use. Researching, monitoring and controlling aspects such as the amount lost through leakage during fog application and breakdown in soil, water and vegetation are paramount to the continuing approved use of the chemical particularly in the potato industry.

The common problem of poor distribution when applied as a thermal fog leads to over use. More chemical than is realistically required for the tonnage of potatoes treated is generally applied throughout the season in an attempt to overcome the distribution issue. This approach is on the whole fairly successful for controlling sprouting however leads to a residue build up in certain areas in both box and bulk stores (*Corsini et al, 1979*). This in turn raises the concern that is currently the main focus of the campaign to optimise and reduce CIPC use. Attempting to lower residues and keep them within the expected Maximum Residue Level (MRL) to be imposed in the UK in the near future. The residue levels depend to some degree on the method of application. Thermal fogging is recognized as a method that while it can be conducted fairly rapidly it does inevitably lead to uneven distribution, with the result of greater variability throughout the store (*Coxon & Filmer,*

1985), particularly when there is a temperature differential most commonly across the height of the store (*Duncan et al, 2001*).

Many in the UK potato industry believe that the MRL will be set at 5ppm to keep inline with current MRL's in most of Europe, although 10ppm would be much more favourable on the basis of values in excess of 5ppm frequently encountered in UK box storage (Dr H. *Duncan, R. Harris, A. Briddon, A. Cunnington, personal communication*). The tolerance residue level in the United States of America is 30ppm. The MRL is measured as the average CIPC concentration on a whole tuber weight basis (from a selected pre-determined sample size) remaining on a potato after being removed from storage at least 21 or 28 days after the last CIPC treatment and following light washing in tap water. Both the time since last application and the washing will reduce the concentration of CIPC on the surface of the tuber to an extent.

At the moment most storage facilities in general have a reasonable percentage of potatoes under the 5ppm concentration, but no doubt they also have the typical top box potatoes that will be in excess of this level (*Burfoot et al, 1996*). For this reason there is research funded by the British Potato Council addressing the issues of distribution and environmental fate (*study 207/197*).

Presently both Tecnazene and CIPC can be used for sprout suppression, although the future of Tecnazene is dubious. This adds to the increasing pressure to improve and develop the knowledge and practical use of CIPC to gain more effective use of the herbicide and strengthen its position in the agricultural industry.

Koivistoinen & Karinpaa, 1965 found that at 10°C approximately 50% of CIPC would be lost in three months from the skin of apples and that generally increasing the storage temperature enhanced the rate of disappearance. The same pattern could also be expected of residues on potatoes. Hence the concentration on potato skin from stores at 10°C would be less than those from 3°C storage assuming an equal amount of CIPC had been applied.

Some examples of residues found on potatoes samples collected from retail outlets are given overleaf. They are government findings from samples of UK potatoes collected in early 2000 (www.pesticides.gov.uk/committees/pvc/fourthq2000/sumtabsq4.xls).

It should be noted that many other samples collected from retailers such as Iceland, Sainsburys and Tesco had no quantifiable residue level of CIPC.

Retail Outlet	Potato Cultivar	CIPC residue concentration
Asda	King Edwards	0.6ppm
Asda	Maris Piper	0.9ppm
Asda	Whites	1.0ppm
Wm Morrison	Estima	3.3ppm
Safeway	King Edwards	2.0ppm
Waitrose	King Edwards	1.2ppm
Waitrose	Sante	5.1ppm

Table 1 Examples of government CIPC residue levels found on potato samples collected from various retail outlets in 2000.

With the exception of one sample all residue levels were easily within the proposed MRL of 5ppm. The higher concentration was only marginally over this level and when it is averaged with the other samples collected in the same period from the same retailer the concentration would more than likely be acceptable under fourthcoming legislation.

The residue of CIPC remaining on potatoes after storage will be much reduced by the home cooking or industrial processing procedure, particularly when fried in oil. The translocation of CIPC into the oil is expected to be quite high, but will vary depending on whether the oil has been used before (*Nagami, 1997*) and the amount of surface area of the tuber flesh and peel that is exposed. When boiled a proportion of the CIPC will transfer into the water.

These processes of residue removal are dependant upon the polarity of the solution used e.g. water is polar, but oil is non polar, the temperature it is conducted at and the extent of agitation.

Peeling, which normally follows, removes most of the remaining residue, although a miniscule amount of CIPC can transfer into the flesh of the potato (*Hajslova & Davidek, 1986; Coxon & Filmer, 1985*). CIPC tends to concentrate in crisps due in part to oil uptake (~35%) and evaporation of water. Thermal degradation is a further potential route for the residue leaving the product as demonstrated by *Nagayama & Kikugawa, 1992*. It involved volatilisation for removal of the degradation product (chloroaniline).

When CIPC is within reasonable proximity to the eyes of the potatoes and in sufficient concentration it provides good control of sprouting for a reasonable period of time. However the considerable drawback associated with application as a thermal fog is the characteristic sugar spike exhibited in the crop following treatment. *Wang & Pritchard, 1997* have demonstrated that application of CIPC as a thermal fog not only generated a sharp increase in reducing sugar levels but also caused an increased rate of respiration. The corresponding darkening of fry colour bears heavily on the prospective retail value of the crop and its overall acceptability in the processing industry. Recovery to previous sugar levels (pre-application) is not always complete. With repeated applications quality can become seriously compromised.

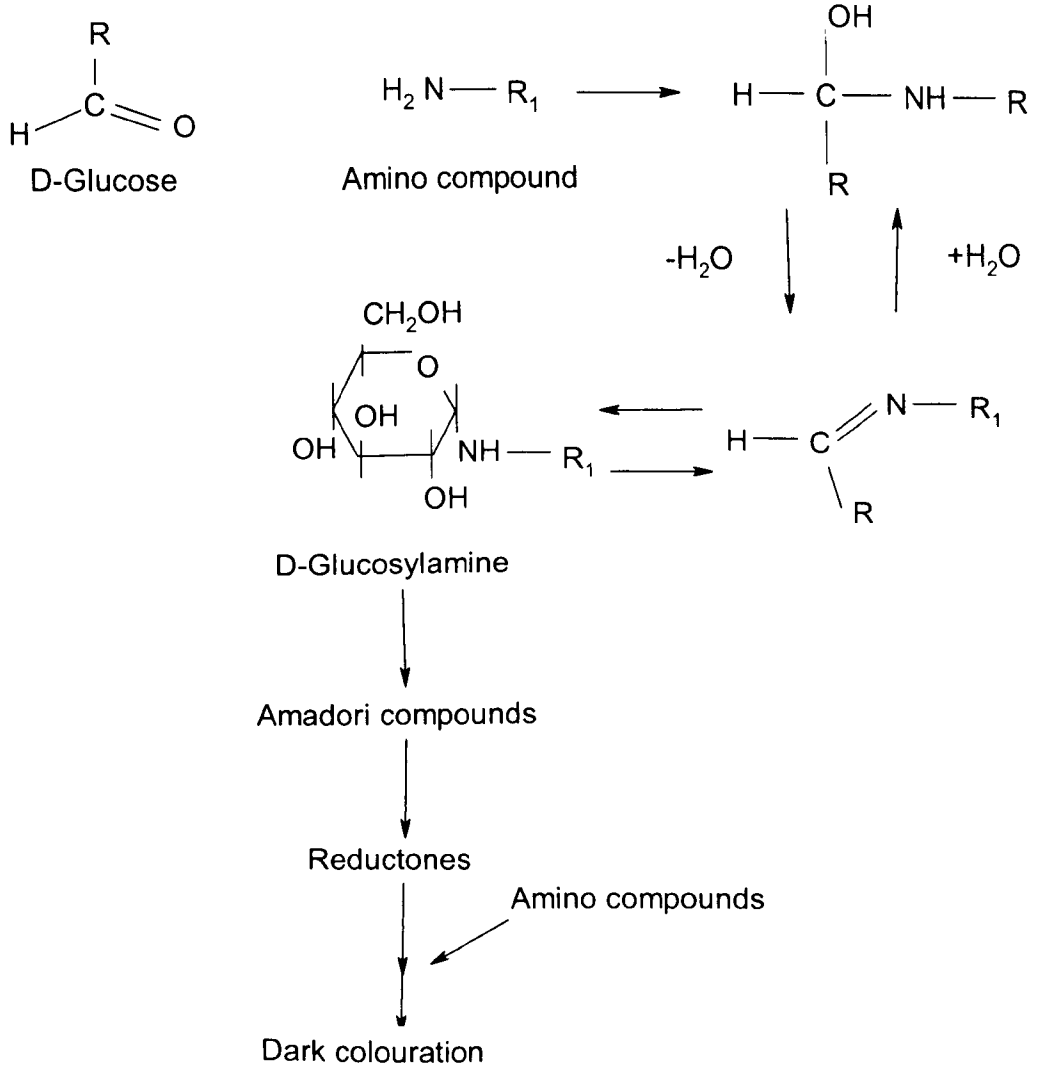
The compound ethylene has been discovered in thermal fogs. This is a gaseous contaminant that is produced on combustion of fossil fuels. Standard thermal fogs are made using the exhaust gases from a petrol engine to heat a high-speed flow of air that carries the CIPC into stores. The suggestion has been made that it is the ethylene constituent of thermal fogs that is yielding the increase in reducing sugars.

This raises many questions: How much ethylene is in thermal fog? What factors influence its concentration? What effect does exogenous ethylene have on potatoes in storage? Is it solely responsible for this deterioration in processing quality? If this is the case what practical options exist to ameliorate this deleterious effect?

1.1.5 Colour development on processing

The undesirable development of dark colour that occurs during processing of potatoes is known as non-enzymic browning or in chemical terms it is the Maillard reaction. As outlined below the reaction is essentially that of the aldehyde moiety of reducing sugars combining with amino acids at high temperatures to produce the dark colour. The reducing sugars combine non-enzymically with tuber amides asparagines, glutamine and other amino compounds to form aldosylamines and sugar reductones, which further polymerise in the presence of the amino compounds. This is how the characteristic brown fry colours and flavours are developed. Not only is the colour not aesthetically pleasing but it is also normally accompanied by a bitter charred taste.

1.1.5.1 Maillard reaction



Although the concentration of the amino compounds can influence the extent of browning, and the contribution of each individual amino acid should be considered (*Beecham & Dull, 1951*), it is generally accepted that the size of the reducing sugar pool is the limiting factor in the development of browning (*Boyd, 1988, Leszkowiat et al, 1990, Roe & Faulks, 1991*). The level of reducing sugars present in the tuber is largely a function of starch conversion and respiration. The amino acids are present from the breakdown of soluble storage proteins and as a constituent of a free amino acid pool (*Brierley et al, 1997*). As a rule potatoes with a high content of reducing sugars give rise to unacceptably dark fry colour and generally the lower the reducing sugar level the lighter the fry colour (*Burton, 1966*). The relationship between lightness of the fried sample and the reducing sugars is thought be linear and sufficiently reproducible (*Marquez et Anon, 1986, Rodriguez-Saona et al, 1997, Uppal, 1999*). Therefore in practise the reducing sugar level of a batch of potatoes can be used as an indicator of the expected processing quality (*Habib & Brown, 1956*). According to *Wunch & Schaller, 1972* the optimum reducing sugar level for a tuber is between 0.1-0.2%. *Duplessis et al, 1996* estimated the ideal value to be 0.1% and more recently the feeling is that levels much closer to zero are desired at <0.005%. The glucose critical value for processing crisps is 0.035%, French fries have a higher tolerance (*Dr M. Kirkman, personal communication*).

When the temperature of processing is excessively high at 220°C and above fry colour can be influenced to a greater extent by total sugar content (sucrose) as opposed to the reducing sugars (glucose and fructose) due to caramelisation. Fortunately the standard procedure for frying crisps has a recommended temperature of 177°C and 190°C for French fries. This project focuses on the production of crisps. There have been many studies into how the Maillard reaction can be altered to limit the extent of browning by the addition of trace metals or ascorbic and citric acids. Other approaches have been to remove some of the soluble sugars by blanching in hot water prior to frying or to stop frying early to prevent moisture decreasing to a detrimental level and then drying the crisps by some other means. Many of these are simply not practical or economically viable for the production of crisps.

Research into industrial frying has shown that the type of oil used and the quality of the oil are important aspects to be considered as they affect the final product (*Melton et al, 1993, Warner et al, 1994*). Factors such as the oil properties, composition (specifically the free fatty acid content), packaging, storage, the frying equipment and process need to be evaluated for the specific end use (*Rossell, 1998*). This applies particularly to potato crisps as typically they absorb a large percentage of oil up to 35 or 40% in weight. Even low-fat crisps have a relatively high oil content. Hence the quality of the oil will become evident

by the quality of the crisps. The issues involved in the frying process, for the most part, can be dealt with by making the procedure uniform and consistent. Normally standardised frying parameters such as time and/or temperature are adopted for testing samples to avoid extra darkening effects arising from the frying procedure (*Sahin, 2000*). In the case of the Standard Operating Procedure (SOP) in place at the British Potato Council's (BPC) research facility Sutton Bridge Experimental Unit (SBEU) the method is designed to include sympathetic conditions (slice thickness, pre-fry rinse, oil temperature, fry time etc) that will produce the lightest colour without forgoing on any other quality such as texture.

Studies have shown that the balance of sugars can be quite different within the different internal sections of the tuber (*Pritchard & Scanlon, 1997*). So the accumulation of reducing sugars and consequent dark fry colour is unlikely to be uniform across the processed product. This is why different categories of sugar defects, followed by an overall colour score is awarded to crisp samples according to the BPC standard operating procedure.

Against this background the following aims were developed.

1.2 Aims

> "Produce acceptability includes a trade-off between price, availability and quality" (*Sloof et al, 1996*)

The price and availability of potatoes for the expanding processing industry are aspects that this project cannot directly influence. However the vital element of quality is the key reason for this research. To keep up with demands, long-term storage of crop at temperatures that require the use of CIPC is fundamental. Although presently Tecnazene can still be used there is strong feeling that it will not be permitted in the next season. Thus CIPC is likely to be the only sprout suppressant available for long-term storage of processing crop in Britain.

The problem with the use of CIPC is the detrimental impact it has on fry colour following application as a thermal fog. During a normal season the number of treatments required is usually more than three to ensure sufficient control of sprouting for the full duration of storage. Even with a pre-harvest treatment of MH, which delays the need for the first post-harvest application, subsequent regular treatment with CIPC is understood to be essential by store managers. This drop in quality, and particularly the repeated drop in quality from subsequent applications can make a considerable difference in the price per tonne and could even be the deciding factor as to whether crop can be sold for processing purposes at all. There is evidence to suggest that the increase in reducing sugars caused by thermal fog application may not be permanent and some recovery of fry colours is possible over time. It is unclear whether the sugar levels fully recover or are incrementally damaged in a more permanent fashion by repeated thermal fog treatments.

There are a number of interrelated issues and concerns highlighted in this review of background literature. The intention is to set the backdrop to the current situation in the potato processing industry, with a view to addressing some of the core problems that appear to be imperative to the continued success of this evolving market.

The main objectives of this thesis are:

To determine precisely the influence that CIPC treatment has on processing quality and elucidate the reasons for this.

To investigate the implied wide-ranging effects of the compound ethylene on potato tubers and clarify whether it is responsible for the characteristic dark fry colour found after thermal fogging.

To find practical solutions for eliminating or reducing the detrimental affect of CIPC thermal fog application.

To achieve the aims both laboratory studies and simulated commercial trials will have to be conducted. Laboratory experiments will focus on the evaluation of fogging equipment and the investigations of ethylene. The storage facilities at Sutton Bridge Experimental Unit are ideal for practical storage trials on a larger scale. This combination of methodical controlled lab work and simulations of actual commercial conditions is the best means of progressing this near-market aspect of agricultural chemistry through research and development.

Any solutions offered or improvements made would realistically have to be simple to integrate and reasonably cheap to make feasible in cost terms.

This type of research is heavily industry linked and the implementation of recommemdations from the project would be expected through this avenue. The involvement of potatoes and potato products in everyday life outlines the underlying importance of continued research in this area.

2 Chapter Two

2.1 MATERIALS AND METHODS

The major proportion of practical work conducted for this thesis employed the same methods of analysis throughout. Therefore a full description of each main method used is given in this chapter. Any specific modifications made to methods for individual experiments have been noted in the relevant chapters. Additional methods or techniques used have also been given in the associated chapters.

2.1.1 Ethylene determination

The preferred method of ethylene analysis is by Gas Chromatography (GC) with a Flame Ionisation Detector (FID). This type of system is favourable as ethylene samples can be injected directly as a gas, they will separate well on a wide range of columns, FID is most suitable for small organic molecules and the sensitivity should be relatively high. A packed size-exclusion column was used throughout the project. This was selected as earlier ethylene work by undergraduate students had been performed in this department using this type of column and the running conditions seemed to be conducive to good separation and reproducible chromatograms at reasonably low levels. Due to the expected workload and constraints on the available time for other equipment, separation using a Capillary or Mega-bore column was not attempted. Retrospectively these may not have been suitable as a high volume of gas injection (up to 2ml) was required to quantify down to low headspace concentrations of samples at around 0.25ppm.

2.1.1.1 First GC

The first GC used was a Shimadzu GC8A (this was this machine used in the undergraduate studies). Time was expended on optimising the analysis of ethylene using this equipment. The aspects that had to be iteratively tested were standard pressurised gases used for calibration and syringe selection (type and range of volumes, Hamilton Microliter gastight syringes). The oven temperature was altered from the previous earlier method as the retention time was considered too soon for satisfactory separation. After the groundwork was completed, the conditions under which ethylene analysis was conducted using this GC were:

Packed Column: Haysep D 100/120 (polydivinylbenzene, Supelco 10293)

Oven temperature: 50°C isothermal

Injector temperature: 200°C

Detector temperature: 200°C

Carrier gas & flow rate: Nitrogen, 27ml/min

Flame gases, air and hydrogen were set for optimum sensitivity and resilience to a high sample injection volume.

A packed column was used for the analysis of ethylene because large sample volumes (headspace gas) had to be injected to allow detection and quantification. A capillary column would have been expected to provide superior resolution but would not have been suitable for injection volumes of up to 2ml. Potentially the flame could extinguish during injection and the column may be overloaded.

The calibration was in absolute units of µl of ethylene. The graph was produced using incremental volumes of standard gas mixtures from pressurised cylinders. The higher concentration range for calibration was done using 100ppm ethylene in helium mixture (100.1ppm in helium Scotty II, Supelco 2-2572) and at the very upper end pure ethylene (99.5% pure, Scott 2001441). The high concentration range was only used to demonstrate that the relationship between volume of ethylene and peak area was linear for the entire range expected to be in use and beyond. For the working concentration range the calibration was done using incremental volumes of 10ppm ethylene in air mixture from a pressurised cylinder (Scotty II, Supelco 501379).

Unfortunately the variability of peak areas between days was quite extensive. This meant that for every day of analysis a new full calibration graph had to be produced. The relationship was always linear and reproducible when the column was used continually, but when long intervals were left between samples i.e. overnight the sensitivity adjusted dramatically. The same calibration graph could be used for an entire day, providing samples were consistently injected. The sensitivity was acceptable for the type of results desired at the time. For day to day analysis a range of Hamilton gas-tight syringes were

used for calibration and sampling. Where larger headspace samples had to be collected a 2ml gas-tight syringe was used.

Methods of collecting, concentrating and transporting samples had to be considered for future trials based at SBEU. Absorbent resins are often used for concentrating airborne analytes (*Alltech Associates, 1998*). Thermal desorption is one way of removing the samples from the resin while avoiding any dilution step introduced by the use of a solvent.

2.1.1.2 Continuous air sampling

Samples collected from large-scale trials outside GU were more problematic because they had to be stored and transported prior to analysis. To do this a fraction of store air was passed through a silanized glass tube containing an absorbent resin to trap the compound.

The glass sample tubes (6mm outside diameter, 3mm inside diameter) had been previously silanized, then packed with a 15mm bed of absorbent resin and a silanized glass wool plug at either end. To silanize, each tube was submerged in a 5% hexamethyldisilazane (HMDS) solution in toluene for fifteen minutes ensuring the entire surface of the glass was in contact with the solution. Upon removal from the HMDS the tubes were first rinsed in toluene then acetone and dried in an oven at 110°C. When dry the tubes could be removed and left to cool in a dessicator until ready to be packed. All sample tubes were conditioned prior to use by heating to approximately 310°C while purged with low flowing nitrogen to remove all traces of oxygen from the resin. When cooled the nitrogen source was removed and the ends of the tubes sealed with teflon tape and aluminium foil. They were stored at 4°C before bringing to room temperature for sample collection. The tubes were used within four days of conditioning to ensure no loss of quality on sample collection or desorption.

The resin used was Carbosieve III (60:80 mesh, Supelco 10293). This was only used in the project since approximately June 2000. Initially the absorbent resin Tenax-TA was tried for efficiency, because it is not affected by humidity or carbon dioxide. Tenax-TA has been used to trap and analyse headspace volatiles for a number of years (*Bunch & Pellizzari, 1979*). It was found that the Tenax resin did not have a high enough affinity for lower molecular weight carbon compounds (*www.sisweb.com/index/referenc, 2000*). This meant that the majority, if not all, of the ethylene in the samples collected passed straight through the resin and was not held.

Continuous air sampling pumps (Aircheck sampler model 224-PCXR8, SKC) with low flow adaptors were used. The sample tubes were attached to these. Where appropriate an additional one-metre length of tubing was added to ensure a representative headspace sample was collected. The extra length of tubing prevented the air that was exchanged with ambient air being sampled (when the system is opened to prevent pressure building up). The sample flow rate is variable depending on the time given to sampling. It is essential that a high volume be collected therefore the sample volume is more important than the flow rate, however the flow rate should never exceed 100ml/minute, and preferably be around 50-70ml/minute. This will prevent any of the analyte simply passing through the resin.

When sampling was complete the resin tube was sealed with Teflon tape, wrapped in aluminium foil and stored at 4°C until transported to GU. The samples taken from stores had to be in a form that could be well maintained while in transit to ensure sample integrity.

2.1.1.3 Thermal desorption

A purpose built thermal desorption unit had been fashioned from a heating block a number of years previous. This block was positioned directly in the top of the injection port of the GC in use for ethylene analysis. The carrier gas inlet was connected through the top of the heating block so that it would sit onto the sample tube and remove the ethylene from the resin when an adequate temperature was reached. Several attempts were made to desorb and collect the ethylene from the resin but with very little success. Temperatures as high as 300^0C are suggested in literature (*Application note 65, Scientific Instrument Services*). Clearly for manageability reasons this has been lowered as much as possible to the optimum. The affinity of ethylene for this resin was so great that a temperature of approximately 280°C was required to desorb it from the Carbosieve (*www.gcms.de/break/cs111hyd.htm, 2000*).

If the release gas from the resin could be collected and controlled while directed onto the chromatography column then it could be analysed for ethylene content. The action of connecting the tube to the carrier gas inlet and the injection port was practically difficult because of the high temperature and the small space available to do it in. Added to this is the time it takes to complete the connection. Immediately the resin is near the heat the ethylene starts to desorb, even without the carrier gas. This causes loss of an unquantifiable proportion of the sample. It would seem obvious to lower the temperature

to lessen this problem, but a cooler temperature would lead to slower desorption and a wider band of ethylene. Unfortunately the susceptible areas in this analytical procedure meant that the output could be skewed unpredictably in either direction. Modifications had to be made to the process to try to improve the control of the gas release.

A valve and extra plumbing was fitted at the front end of the GC as part of a system that would allow the carrier gas to be diverted through a loop. The heating block was set up around the new gas lines and the length of the sample tube was extended for easier and faster connection. This arrangement meant that carrier gas was flowing through the GC column while the sample was prepared in the extra section of plumbing. The sample was connected to the preheated block and the gas lines fixed to the top and bottom of the tube. This still had to be completed rapidly to prevent loss due to fast desorption because of the heat.

As soon as the connections are complete the position of the valve is switched and the carrier then flows through the sample tube bringing the desorbed ethylene onto the top of the column. Only a fixed volume from the sample line was transferred onto the GC column and this dependant upon the length of stainless steel tubing used for the loop. The diameter of the pipe was fixed so only the length contributed to the capacity. Initially a 5µl loop was used but quantification of ethylene was not possible because only small and inconsistent peaks were detected. The timing of the switching of the valve was altered in stages to cover all possibilities in an attempt to get repeatable standard peaks from this desorption technique. Following this the volume of the sample loop was increased to 20µl. This improved the collection of the correct fraction of the gas exiting the sample line and permitted standardisation of the time after connection when the valve was switched over. It did not however allow adequate calibration of ethylene at any concentration due to variability.

Even with the set time for valve operation and a more suitable loop volume the error was too large and inconsistent and consequently could not be factored into the analysis method. The variability was associated with samples loss because the resin tube had to be connected through the heating block before the gas line circuit to the valve could be connected.

It was not possible at this point to afford this system further time and resources for development without sacrificing in other areas of the project. For this reason a semi-quantitative method of ethylene determination was used in samples to be collected from

experiments outside GU. A different method described further on was used for desorbing and quantifying concentrated resin samples that were collected at GU later in the project once time had become available to reassess the procedure.

The semi-quantitative method of determination for ethylene concentration used in SBEU stores is the Gastec system. This system is compared with GC analysis in the correlations chapter. Gastec quick-determination gives a concentration range of ethylene and the higher the concentrations the wider the range. Full details are given in the following chapter.

2.1.1.4 Second GC

The condition of the Shimadzu GC had continued to deteriorate and it had to be disconnected and abandoned. It was replaced with a PYE Unicam PU4500 with FID and Spectra physics SP4290 integrator. With this machine the running conditions for ethylene analysis were:

Packed Column: Haysep D 100/120 mesh (polydivinylbenzene)

Oven temperature: 50°C isothermal

Injector temperature: 200°C

Detector temperature: 250°C

Injection volume: variable (10µl to 2000µl)

Carrier gas & flow rate: Nitrogen, 30ml/min

Air and hydrogen were set for optimum sensitivity and resilience to a high sample injection volume.

The calibration was exactly the same as with the first GC and a similar problem occurred with full calibration being required on a daily basis. Even with this time consuming procedure the method was sufficiently reproducible for daily analysis of ethylene gas samples.

2.1.1.5 Alternative thermal desorption

After sampling the Carbosieve SIII absorbent resin (ethylene traps) was removed from the glass tube and placed inside a 5ml high-pressure headspace vessel. The vessel had a septum lid to allow gas samples to be extracted using a gas tight syringe. When ready to sample a vessel was placed inside a metal holder on top of a hot plate. It was heated to 220°C and held at this temperature for 5 minutes to desorb the trapped ethylene, then 2ml of headspace was collected and injected into the GC for analysis. The results were calculated on the assumption that the headspace inside the vessel was at equilibrium when sampled and therefore of a uniform concentration. Secondly, that all the ethylene had been released from the resin. Preliminary work on the absorption and desorption technique had been carried out to find the optimum conditions of temperature and desorption time. The temperature used could be lowered because the time allocated for desorption was increased. The time could be increased because the headspace vessels were closed and no loss of sample could occur. The method was calibrated by way of desorbing and sampling resin previously spiked with specific concentrations of standard ethylene gas under the same conditions used for samples. This method proved successful for use in experiments when a concentrating step was required and the resin tubes could be desorbed within two days. This method was not in place until the latter stages of the project and as such was not tested for use in conditions that meant samples would have to be stored and transported for period of up to a week or ten days before analysis. It is expected that it would have been satisfactory for this type of sampling.

2.1.2 Analysis of carbon dioxide gas

2.1.2.1 At SBEU

Carbon dioxide analysis was carried out by means of a landfill gas meter that has a limit of quantification of 0.1% CO_2 (0.1% equating to 1000ppm). This was regarded as sufficiently sensitive for determination of CO_2 in large store environments. This system was used in trials conducted in 12 tonne experimental stores at SBEU and is a good gauge of larger differences in composition of individual store atmospheres. It was also used for any sampling done in commercial stores. It was very suited to the purpose of these trials.

2.1.2.2 At GU

For more detailed investigation of the major components in a simulated store atmosphere and their relevant interactions a more sensitive monitoring programme was required. Methods of increased sensitivity are much more conducive to laboratory surroundings than a potato store. So, for detection of subtle differences in small-scale experiments CO_2 was measured using an alkali trap, which was then back-titrated with an acid solution. From this the amount of CO_2 in the trap could be calculated and the information extrapolated to correspond to overall store levels. In this method there is no distinction between CO_2 from respiration and any applied exogenously. It all behaves the same in the alkali and subsequent treatments.

For passive sampling over an extended period for cumulative CO_2 measurements (in most cases one day or one week) the 1M NaOH was placed inside a brown glass wide-neck bottle. When sampling started the bottle was opened. When sampling was complete the bottle was sealed and stored until it could be titrated. The volume of alkaline solution depended very much on the duration of sampling and the expected rate of respiration. Excess solution was always used to be sure that the trap would not become saturated with CO_2 and hence ineffective before sampling was complete.

When the above method was not suitable smaller gas samples were extracted from the storage containers using either gastight or luer-lock disposable syringes. A silicon tubing extension into the centre of the container was always used. This was to ensure that the sample was representative of the potatoes and not only of the air near the top of edges of the storage facilities. It is assumed that the air around the potatoes is uniform in composition.

Three blank samples were included with each batch to be titrated. This accounted for any background CO_2. The mean blank value was subtracted from each sample titre volume before calculation. Immediately before titration each alkaline trap was opened and an excess of 1M $BaCl_2$ solution was added along with 3 drops of 1% phenolphthalein indicator. The concentration of the hydrochloric acid (HCl) used to titrate the sample depended on how much was expected to be in the trap. The more CO_2 in the solution the more acid was required to neutralise the sample. Where sampling had been conducted over a relatively long period (seven days) 1M HCl was used, and when small portions of air were sampled then 0.01M HCL was used and similarly intermediate time scales required 0.1M HCL. The selection of acid concentration was important in having control at the point of colour change so that more precise titre volumes were obtained.

The volume of acid required to neutralise the sample equated to the weight of carbon present as carbon dioxide (1ml 1M HCl=6mgC-CO_2).

2.1.3 Fry colour assessment as an indicator of potato processing quality

This measurement includes the procedures of sample collection, preparation, frying, storage, visual crisp assessment for defects and Potato Chip Snack and Food Association (PCSFA) scoring and assignment of a Hunter Lab value. All of which are described in the following section. The methods detailed are the standard operating procedure employed for processing quality assessment by fry colour at SBEU. They are detailed as found in the BPC work procedures available at SBEU.

Each sample was subject to an identical procedure and all samples fried on the same day were assessed on the same day, although this could be after a period of storage (up to approximately four weeks).

All fry samples (excluding those fried at GU) were processed at SBEU. This was done by me and the laboratory staff at SBEU. With the exception of the early samples A. Jina (BPC) and I completed all crisp assessments. A. Jina and G. Wright carried out the first series of assessments. When time was available the methods were explained and demonstrated to me by A. Jina, then performed under his guidance until he was satisfied with my judgement.

2.1.3.1 Potato Laboratory Fry Procedure

The purpose is to check the suitability of tubers for processing into good quality crisps, by Product Appearance Evaluation (P.A.E.) and colour.

Sample preparation

A randomly selected sample of thirty tubers was collected from each sample tray. These thirty tubers constitute a single representative replicate of each treatment. Each sample was peeled until 95% of the peel was removed, using a Hobart 'rumbler' machine with a constant flow of water. The machine has abrasive walls and an abrasive contoured base that spins during operation. This combination of features removes the peel from the potatoes. The longer the sample is left inside the machine the more peel is removed.

Method

The peeled tubers were cut in half longitudinally using a guillotine. An armoured glove had to be worn while doing this.

Rutland slicer OM220SR calibration

Four spare tubers were cut in half longitudinally. Each potato half was placed in the slicer and three slices removed and discarded. A further three slices from each tuber were removed to give at least ten slices. The thickness at three points around the centre of each slice was checked using the Mitutoyo gauge and the three results averaged. The overall average had to be 53 +/- 1 thousandth of an inch with a range of less than 9 thousandths of an inch. If the slices were out of the specification range the thickness dial on the slicer unit was adjusted accordingly and the thickness rechecked.

Mitutoyo slice thickness gauge 7300 calibration

The lever on the side of the thickness gauge was pressed to lift the measuring piston off the rest. A 0.050inch standard gauge block was inserted between the measuring systems and its rest. The measuring piston was slowly lowered on to the gauge block and checked to ensure it was properly seated between the piston and the rest (tolerance of the thickness block is 0.050" +/- 0.001inch to be accepted and applicable for use in the frying procedure).

Preparing slices for crisping

A half potato was placed in the previously calibrated slicer. The first three slices produced were discarded. A further three slices were removed and checked for thickness. If the slices were within specification they were placed in a plastic tray for weighing. If they were not within specification, the slices were discarded and the slicer was recalibrated. This process was repeated for each half tuber until 300g of slices per sample had been collected. The digital balance used was previously calibrated with a standard 5kg weight.

Every 300g sample was washed in cold water for forty-five seconds in a plastic bucket, with continuous stirring. The excess water was shaken off and the slices placed in the fryer basket and covered with the submerger, to prevent slices floating off during frying.

Frying the crisp samples

The vegetable oil in the fryer (Bartlett fryer D11E 30) was heated to 177°C. This temperature was monitored using a digital thermometer with a suitable probe attached. When 176°C was reached the power to the fryer was switched off immediately and the basket placed in the oil. Safety glasses were worn during this procedure. The temperature must be achieved by the described method. The temperature profile is incorrect if the oil is overheated and cooled to 177°C. The oil must be heating to the correct temperature when the slices are submerged.

The samples were fried for three minutes. The basket was moved vigorously in the oil to prevent the slices sticking together for the first and last minutes of frying. After this time the samples were removed from the oil and allowed to drain and cool.

No more than fifty samples were fried in the one batch of vegetable oil before the fryer was emptied cleaned and refilled with fresh vegetable oil.

Storage of samples until assessment

Crisp samples were transferred into plastic food storage bags and the bags tied after as much air was removed as possible without crushing the crisps. The bags were put into a communal black plastic bag to prevent any damage due to light. The black bags were then placed inside a cardboard box to protect the samples. The boxes were labelled and placed into a 3 tonne potato store at SBEU at 10°C for storage until assessment could be carried out.

2.1.3.2 GU frying process

The frying process was scaled down to ensure that all samples could complete in a reasonable time scale. They also had to be manageable in the smaller slicer (Kenwood Food slicer SL250) and fryer used. Ten tubers were used per sample instead of thirty. Each tuber was halved longitudinally. A number of slices (approximately three) from each half tuber were used to make the required 100g sample weight. The slices had to be within the thickness tolerance of 1.12-1.57mm. The tolerance range equates to 53 +/-1 thousandth of an inch with a tolerance of less than 9 thousandths, the same as the range used for the frying procedure at SBEU. This was measured using an analogue set of callipers (Altec Economy Dial Calliper). The slice that took the weight of the sample over

100g, using a top pan balance, was the last slice to be included. They were washed in flowing cold water for forty-five seconds then drained and placed inside the frying basket avoiding the edges to prevent the slices escaping during frying. A wire basket-type lid was placed over the slices. The rest of the frying procedure was identical to that used at SBEU for crisping. The digital thermometer used to monitor the temperature of the vegetable oil prior to the start of frying was an Altec testo 925 with a thermocouple type K (Ni-Cr-Ni) immersion probe with a working range of -60°C up to 400°C.

2.1.3.3 Product Appearance Evaluation

Method

Visual crisp assessments were carried out inside a light cabinet on a white surface using the natural daylight setting. The sample was first weighed using a digital balance that had been previously calibrated and checked with a standard 5kg weight. Defects were determined and removed in the following order of priority:

Green – A green discolouration showing at the edge of the product. Caused by exposure to sunlight resulting in chlorophyll and leaving a bitter flavour.

Purple – A purple discolouration, generally on the inside of the product

Undesirable colour – All dark areas of the crisp i.e. blue, black or brown (due to high sugar content) must be added together to produce an area of at least 50% of the crisp and have a Hunter L value of less than L49. Caused by the Maillard reaction resulting in brown crisps with a burnt flavour.

Internal defect – Any defects not touching the edge of the crisp, which adds up to an area of at least 5mm in diameter and that are darker than a Hunter Lab value of L49. Internal defects are further categorised into sugar defects and internal bruise. The sugar defects are Pith, Heel, Rose, Vascular, Matrix and Spot. All defects have to be larger than the template (5mm) and darker than L49 when added together to be classed as defects. Internal defects are differentiated from Undesirable colour by size, and from external defect by location, and they are not detectable prior to slicing. Although generally this group does have the same causes and it is perceived by consumers to be foreign to the natural appearance of the crisp.

External defect – Any defects that touch the edge of the crisp and are greater than the size template (5mm) when added together. External defects are also split into Sugar and Bruise defects, each of which has to exceed 5mm in diameter. They are caused by a variety of diseases and handling practices (e.g. mechanical damage, bruising). Again they are perceived by consumers to be foreign to the natural appearance of the crisp.

At each stage crisp defects as listed were weighed and discarded. The remaining crisps were deemed as acceptable and given a general PCSFA score (*Fick & Brook, 1999*). The sample was then retained to obtain Hunter Lab values.

The PCSFA score is a subjective value attributed to the general appearance of a crisp sample. Colour reference cards are used to illustrate the score categories. Using these as a guideline a score of 1, 2, 3, 4, 1-2, 2-3, or 3-4 was assigned. Each score can be related to an approximate Hunter Lab value. These values are to ensure this subjective assessment conducted by me or on occasion by Ajay Jina of SBEU, were a good reflection of the automated value gained by using the Hunter Lab system. PCSFA scores and Hunter Lab values for all samples corresponded well. The approximate L values for colour grades are (PCSFA = Hunter Lab value) 1=65, 2=62, 3=58, 4=55 and 5=49. Please see the colour chart in *chapter 3* for an illustration of these values.

2.1.3.4 Hunter Lab Assessment

Information from the Manual and brochure associated with the Hunter Lab Instrument (*Hunter Associates Laboratory, Reston, VA 20190*).

Background

Appearance characteristics are very difficult to communicate objectively and the fact that colour is a three dimensional characteristic makes it particularly challenging. Colour consists of a lightness attribute called 'Value' and two chromatic attributes called 'Hue' and 'Chroma'. Colours can be distinguished from each other by specifying these three visual attributes. Figure 3 below displays a common arrangement of these attributes often termed "colour solid" or "colour space".

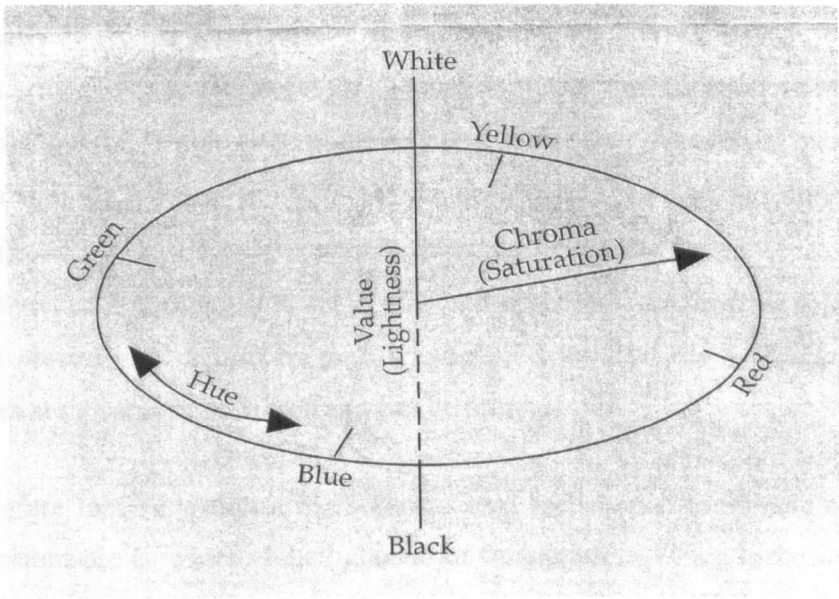


Figure 3 Three-dimensional colour coordinate system

Hue is the attribute of colour perception by which an object is judged to be red, yellow, green, blue and so forth. Chroma is the accepted term used to specify the position of the colour between grey and pure hue. Value is the term commonly used to express lightness and is a measure of light reflectance which distinguishes 'light' colours from 'dark' colours.

The L-value attributed to a sample equals 10 $\sqrt{R}$, where R equals the percentage of total incident light which is reflected.

This automated, scientific system for specification of colour was designed to be a) objective, rather than have to rely on subjective human judgement and b) reproducible by excluding variations in colour vision as potential sources of error.

Design

The system had to be checked using a sequence of calibrated coloured tiles. The details for which can be found in *Annex A of the Quality management system, work procedure of BPC SBEU.* This colour check was done every four hours when the unit was in use consistently for a long period of time.

The Hunter Lab unit consisted of both a D25 optical sensor and a DP-9000 processor. In the D25 optical sensor, light from a quartz halogen cycle lamp is directed at the specimen at an angle of 45° from the perpendicular. The reflected light is then collected in a receptor located directly above the specimen at 0° from the perpendicular. The electrical signals in the receptor are directed to the processor. The sensor has a viewing aperture in the front of the cover to allow the sample to be accurately positioned over the measurement port. The specimen area of the L sensor was 95mm in diameter. The specimen was illuminated from all sides. The DP-9000 processor converts the signals from the optical sensor into colour values relative to the C illuminant and 2° standard observer (*standardized sources of light and human observer as defined by the Commission Internationale de l'Eclairage, CIE*). These values are displayed on screen and can be printed.

The colours are located within a three-dimensional rectangular coordinate system. The three dimensions are L, which is the lightness of the sample, a which is the scale of red to green and the b which is the scale of yellow to blue.

Method

The crisp sample was placed into the round dish and the disc provided was used to crush the sample inside the dish. The sample must be flush with the sides of the dish. This was done to ensure a flat surface was offered to the porthole of the Hunter Lab. At this point the first of three L values were recorded. The dish was then rotated 120°C to take the second reading and this was repeated to take the third reading. Once all three readings were taken the sample was discarded and the dish was cleaned in preparation for the next sample.

The average of the three readings was taken to represent the mean Hunter Lab L value assigned to the sample. It is this value that was used in calculating the mean of all of the treatment replicates performed throughout the course of this project. This is a measurement of the lightness of the sample.

2.1.4 Sugar analysis

Various methods of sugar analysis were researched and considered before a satisfactory method was decided upon. Firstly the Phenol Sulphuric method which required rigorous levels of cleanliness and to some extent isolation to perform. Any stray dust particles could greatly influence the intensity of the colour developed. Initial assessment of the method established that it would not be suitable for fast throughput of raw potato samples due to significant contamination interfering even with calibration standards.

The choice not to use the Technicon enzymic method based on the Boehringer method involving colorimetric measurement of each individual sugar was based on the extraction procedure. A high number of samples had to be extracted fairly rapidly to prevent the influence of ambient laboratory temperature affecting the results. This required a fast extraction procedure that could be conducted solely by one person. The additional step of cooling a large proportion of the solvent in solid carbon dioxide lengthened the process, particularly because the dry ice would have to be refreshed several time during each sampling occasion. Another feature that led to the decision not to use this method was the quantity of solvent needed per sample. 500ml was used to prepare each sample and then some to rinse the glassware in between samples. Compared to the Roe methods and others outlined below this was five times as much solvent for every sample. For the high sample numbers this was not sensible or justifiable.

Ideally the method used would permit simultaneous analysis of glucose, fructose and sucrose for maximum efficiency. GC would have done this, but constraints on the availability of equipment meant that while ethylene analysis was required this method was not an option. High pressure liquid chromatography (HPLC) would also allow simultaneous analysis of these three sugars. An HPLC system was assembled using a lead exchange column, isocratic separation in a methanol solution and an infrared detector. The performance of the system was disappointing even after steps were taken to try to improve the separation and detection like adjustments to pump flow rate, concentration and choice of mobile phase, matrix of the sample, injection volume and optimising response from the detector. The most achieved was very broad peaks that no doubt masked smaller peaks within them. This technique was discarded in favour of the methods described below, which up to this point has worked quite well although were time consuming.

Fructose (free and derived from sucrose) was determined by the Roe method (*Roe, 1934; Jarvis et al, 1974*). Fructose solely derived from sucrose was measured by the Leloir-Roe

method (*Cardini et al, 1955; Jarvis et al, 1974*) and glucose plus fructose was determined using the Somogyi-Nelson method (*Whistler Wolfrom, 1962*). All colorimetric analysis was performed using a Hitachi V-1100 Spectrophotometer.

Commercially a Yellow Springs Instrument (YSI) sugar analyser is used and this seems to correlate well with spectrophotometric methods (*Mazza, 1983*). Spectrophotometry is still widely used but this is normally within the soft drinks industry. Current systems are heavily automated and rely on the method of standard addition to avoid sample matrix problems (*Canizares et al, 2001*).

Tubers samples were washed, peeled, chopped finely and a representative sub sample of approximately 25g was extracted by homogenising with 60ml methanol in a Waring blender. Care had to be taken that each sample included a range of tuber tissues and not only from the stem or apical end. *Pritchard & Scanlon, 1997,* showed that glucose and fructose levels alter between the two ends and that no specific section of the tuber could be assessed to represent the whole tuber. The extracts were filtered through Whatman no.1 paper under vacuum and made to 100ml in a volumetric flask. Dilutions in water or methanol were prepared as necessary for analysis. The matrix had to be matched in every case between samples and standards. In the case of the Leloir-Roe method, on addition of the acidic reagent (B) the sample became cloudy and could not be used in spectrophotometric analysis. These dilutions had to be made in water and the matrix matched for standard preparation. The most common dilution factor required was 1/20, which eliminated the development of cloudy samples. Where water is referred to ultrapure water (~17ohm resistance) was used to avoid interference with colour measurements. An appropriate number of blanks and check standards were included with each batch of samples analysed.

Blood sugar test strips were tested as a rapid method for determining approximately the concentration in the extract. This was done to try to reduce time spent on analysing extract repeatedly until the correct dilution was found. This approach was used in work by *Coleman et al, 1993* who demonstrated that a blood glucose monitor was useful for quick field assessment of expected fry colour of chips. It was well correlated over the acceptable range of fry colour and corresponding glucose content. Similarly a good correlation between HPLC or multiwell ELISA assay analysis and blood glucose test strip has been demonstrated (*Mann et al, 1991, Misener et al, 1995, Pritchard & Adam, 1994*). Unfortunately this approach was not successful with the blood test strips used at GU

because the extractions were in methanol and all the dilution factors had to be arrived at the long way round.

2.1.4.1 Roe method

Reagents

0.15% Resorcinol (Sigma R-5646) in ethanol (A)

7.5mg ferric chloride in concentrated Hydrochloric acid (B)

Method

3ml of sample was added to a boiling tube. 4ml of reagent A and 5ml of reagent B were pipetted into the boiling tube also. A glass marble was placed on top of the tube and the tube was placed into a water bath preset to 77-79°C for 30 minutes. After this time the tube was removed and placed into a cold water (tap water) bath for 10 minutes to allow the colour to develop. An aliquot of the sample was transferred to a clean plastic cuvette and the absorbance was read at 460nm.

The amount of fructose was determined by comparison with a standard fructose graph in the range 0-80mg/l that was produced using this method (Sigma F-D127).

2.1.4.2 Leloir-Roe

Reagents

Reagents A and B were the same as with the Roe method plus:

1M Sodium hydroxide (NaOH). Made using pure pellets (Analar 102525P)

Method

In this method adding 1.5ml of 1M NaOH to 1.5ml of the sample destroyed the free reducing sugars in the sample. The sample was placed in a water bath at 100°C and heated for 10 minutes. When cooled the Roe method as described above was carried out on all samples and they were measured colorimetrically at 460nm.

Again the amount of fructose derived from sucrose was determined by comparison with a standard fructose graph.

2.1.4.3 Somogyi-Nelson

Reagents

Reagent A: 12g potassium sodium tartarate (Analar 102194Q) and 24g anhydrous sodium carbonate (Analar 102404H) were dissolved in 250ml of water. 4g copper sulphate Analar 100914Q) (in a small quantity of water) and 16g of sodium hydrogen carbonate (Riedel-de Haen 13433) were added with stirring. A solution of 180g anhydrous sodium sulphate (Analar) in 500ml was boiled to expel air. These two solutions were combined and diluted to 1l. After standing for two days the combined solution was filtered (Whatman no 1 paper) then filtered again a further two days later.

Reagent B: 25g ammonium molybdate (Analar 100284J) was dissolved in 450ml of water and 21ml concentrated sulphuric acid was added. A solution of 3g disodium hydrogen arsenate (Sigma A-6756) in 25ml water was prepared and added to the acidic solution. Once combined, reagent B was diluted to 1l and stored in an incubator (LMS cooled incubator) at 37°C until brought to room temperature prior to use. Storage at this temperature was critical to the maintenance of the reagent.

Method

1ml of reagent A was added to 1ml of sample and heated in a boiling water bath for 20 minutes. To prevent loss of sample a marble was placed on top of the boiling tube during the 20 minutes. When cool enough to be handled 1ml of reagent B was added to the sample then the tube was immediately placed onto a vortex genie to mix thoroughly. Care had to be taken to prevent spillage of sample while doing this. The sample was made to 10ml with water and mixed again then sonicated in an ultrasonic bath for a few minutes to clear all remaining bubbles. An aliquot of the sample was transferred to a clean plastic cuvette and the absorbance was read at 500nm.

Although 690nm has been used in the past for absorbance readings 500nm was selected because it gave the most reproducible standard graphs, even though it was not the maximum absorbance.

The amount of reducing sugar (glucose plus fructose) was determined by comparison with a standard fructose graph in the range 0-80mg/l that was produced using this method (Sigma F-D127). Initially both a glucose and fructose standard graphs (0-80mg/l) were prepared to verify the response was equal for each. The selected sugar used for daily calibration when sampling was fructose, but glucose would have served just as well.

2.1.5 Auxiliary materials and methods used throughout the project

2.1.5.1 CIPC applications at GU

Solutions for applying CIPC were made in methanol at 50% w/v like the main commercial formulation used in larger scale trials at SBEU. This was applied using a heated air stream (~200°C) into which the formulation was delivered and atomised before being deposited onto the surface of the tubers. This procedure was designed to emulate as far as possible the CIPC applications performed in the larger experiments. The distribution was better with this method because it was on a smaller scale and there was more physical control of the application pipe.

A dust formulation was used for one item of work. This was a 1% concentration of CIPC on an aluminium oxide support material. A solution of CIPC in acetone was prepared and mixed through the pre-weighed dust in a large crystallizing dish. The dish was placed in a fume hood at approximately 18°C to evaporate off the solvent without losing any substantial amount of the CIPC. Total evaporation took about 30 hours. The dust was applied to tubers at a rate of 2g/kg via a hand held shaker with multiple small openings yielding fairly good distribution.

2.1.5.2 Measurement of sprouting

This was done subjectively by eye. All assessments were carried out by me and therefore any bias would have been consistent. A metric calibrated ruler with divisions of 1mm was used. Treatment effects were classified as the mean length of the longest sprout (mm).

2.1.5.3 Additional equipment used at SBEU

An anemometer to measure the flow rate output from a thermal fogger machine.

A digital thermometer to measure temperature of air exiting the application pipe of a thermal fogger and the oil temperature during the frying process.

Both of these items are registered at SBEU and have a logbook of use and regular calibration and quality control testing.

2.1.5.4 General potato cultivar information

The two cultivars used throughout all of this experimental work were Saturna and Cara. Saturna was used in trials where the main objective was to define clearly the effect on processing quality i.e. When frying and/or sugar analysis were the crucial results. When Saturna was not available cv. Cara was used. This was done acceptable in trials that were focusing on the effects of ethylene on sprout growth and assessing additional characteristics such as volatile organic compounds. For this work it did not matter that ethylene exposure caused higher sugar levels because that was not critical to the outcome of the experiments.

Saturna is a common crisping potato and a good indicator of how general processing crop would behave in similar conditions. It has a medium to long dormancy period and a high dry matter content. Its flesh is yellow in colour.

Cara is a table or baking potato with a creamy coloured flesh. It has a medium to long dormancy period and a low dry matter content.

These two cultivars provided a good balance of both pre-pack and processing potatoes for representing specific fields of commercial operation.

2.1.5.5 CIPC extraction and analysis

Although many extractions were performed during the time period of this project only a few samples have been conducted as part of the work presented here. They were to verify that applications made during experiments were successful. The standard procedure of solvent extraction (~120ml hexane) using Soxhlet reflux for 2hours with anhydrous sodium sulphate to remove water was used. Normally a representative subsample of 25-30g is selected for extraction from each sample set, and in duplicate or triplicate as appropriate. Samples were then rotary evaporated down to ~1ml and quantitatively made to 2ml in a volumetric flask.

These extracts were analysed by GC with FID detector (PYE Unicam PU4500 with Spectra physics integrator or occasionally Hewlet Packard 5890GC). The operating conditions were:

Packed column: 3% OV-17

Injector/detector temperature: 250°C

Oven temperature: 180°C isothermal

Carrier gas & flow rate: Nitrogen ~30ml/min

Injection volume: 5µl

The GC was calibrated with a 100ppm standard in hexane. The response is linear over the working range and far beyond. This is the standard routine method used for residue samples of commercial samples from within the potato industry.

2.1.6 Statistical Analysis

Sample standard deviations, calculated using the Microsoft Excel™ statistical function, were used to provide an estimate of variation. This was adequate because the number of treatment replicates within experiments was the same and therefore all comparisons were done with equal sample sizes. The sample size (n) is stated in all cases. Plus and minus one standard deviation is presented on the bar graphs as error bars. This is the conventional manner for reporting results in BPC/SBEU documents.

Further to this Analysis of variance was carried out using a Minitab™ package and Tukey's pairwise comparison found in the One Way Anova function. The confidence interval was set at 95% for all analysis. The Anova values indicated whether or not there was a significant difference between treatment means. Letters were assigned to the mean of each treatment. Where the letters are different there is a significant difference at $P<0.05$. An example is given below.

This is an example from the correlation trial data presented on page 64. There were three replicates per treatment, therefore n=3 in all cases. These are the findings from Day 14 of a storage trial comparing the effect of storage temperature on fry colour (Hunter L-value).

One-way ANOVA: Day 14 versus Treatment

```
Analysis of Variance for Day 14
Source     DF        SS        MS        F        P
Treatmen    2   100.068    50.034    94.05    0.000
Error       6     3.192     0.532
Total       8   103.260

                                    Individual 95% CIs For Mean
                                    Based on Pooled StDev
Level       N      Mean     StDev  ---+---------+---------+---------+---
cold        3    54.360     0.295   (--*---)
optimum     3    61.710     1.002                          (---*--)
warm        3    61.120     0.711                        (---*--)
                                    ---+---------+---------+---------+---
Pooled StDev =    0.729             54.0      57.0      60.0      63.0
```

```
Tukey's pairwise comparisons

    Family error rate = 0.0500
Individual error rate = 0.0220

Critical value = 4.34

Intervals for (column level mean) - (row level mean)

            cold         optimum

 optimum      -9.1776
              -5.5224

 warm         -8.5876      -1.2376
              -4.9324       2.4176
```

The confidence intervals are stated in the Tukey's data for each comparison. If the two intervals pass through zero then there is a chance that the difference between the treatment means could be zero, hence there is no significant difference between this set of treatments. However if the intervals do not pass through zero then there is a significant difference between the selected treatment means.

On this occasion the Cold treatment is significantly different to both the Optimum and Warm treatments, but the Warm and Optimum treatments are not significantly different to one another. This is denoted in the following table by the use of different letter for significant differences.

Treatment	Day 14
Cold	a
Optimum	b
Warm	b

Table 2 Example of statistical analysis (Day 14 results from correlation trial (p64)

This technique has been applied throughout for stating significant differences in all experiments. The analysis of results was all done on an individual day basis, hence the statistical comparison of treatments is within days and not across the duration of the trials. Although it would have been preferential to examine the effects over the entire time period this was not possible. Assistance was sought from a statistician, Chris Dyer, and attempts were made at parallel line analysis (results included in relevant chapters) however any useful outcomes were limited. He recommended that analysis be confined to within day comparisons and trends across the storage period be discussed in general terms. It was agreed that this was appropriate considering the nature of the research and the difficulties of replicating treatments in controlled storage environments on a large scale.

3 Chapter Three

3.1 CORRELATIONS FOR METHOD VALIDATION

Throughout the course of the project different methods were used for analysis of ethylene concentration and determination of the quality of the potatoes. The selection of a given method depended mostly on the practicalities of where the experiment was performed and what equipment was available. In some cases methods had not been fully developed at the time the trial work began and therefore alternative methods had to be adopted in the interim period to allow the project to progress. The results of all the practical work conducted have been compiled and evaluated in this thesis. To justify the inclusion of results derived from different methods the relationship between them had to be evaluated. Assessments of the applicable methods were made and correlation graphs prepared to show how well if at all the methods corresponded.

3.1.1 Systems of detection used for ethylene analysis: Gastec compared with Gas Chromatography

The Gastec colorimetric system was used in all trials conducted in the experimental stores at SBEU. They are glass tubes containing a reactive bed, which on exposure to ethylene produces a blue colour. The length of the reactive bed that changes to blue depends upon the amount of ethylene passed over the bed. Hence when the fixed volume of sample gas (400ml, at 50ml/min) is drawn through the tube using the hand held pump the concentration in that sample is given by the markings on the tube indicating the length of blue bed that equates to a specific concentration in the 400ml.

Figure 4 Gastec colorimetric detector tube for ethylene analysis in the concentration range 0.2-50ppm

The tubes used are for an ethylene sample concentration of between 0.2-50ppm. However the higher the concentration the wider the bracket of concentration range indicated by the blue colour in the bed. At the lower end the concentration brackets are 0.2-1.0ppm, 1.0-5.0ppm and 5.0-10.0ppm, after this the brackets widen to become 10.0-30.0ppm and finally 30.0-50.0ppm. Thus, the tubes are assumed to be more accurate towards the lower end of this range of concentration, and the optimum would be within the 1.0-5.0ppm or 5.0-10.0ppm brackets to keep well within the working range of the colorimetric tubes.

The precision of the result is questionable because of the subjective nature of reading the value from the detector tubes. Added to this is the fact that the reading produced was quite often not neat and seemed to tail slightly, sometimes fading into the adjacent concentration bracket. There are brief notes supplied with the equipment suggesting how a result should be determined in this situation. The literature states that when the blue colour ends in a slant the value in the middle of the slant should be taken as the reading. If the demarcation of the colour change layer is pale, the value in the middle between the pale and the dark end should be taken (*Anachem, Gastec operation manual*). Generally these guidelines helped in determining the ethylene concentration in store atmospheres during trial work, except where methanol was used as a fuel source for thermal fogging when completely diffuse colour the entire length of the reactive bed occurred.

This colorimetric system was deemed to be suitable for use in the trial work conducted. It gave a good indication of what the concentration range of ethylene was in stores as a result of different fog treatments. The concentration divisions marked on the tube were narrow enough to highlight any real differences between treatments applied. The use of this system eliminated the need for concentrated collection, transportation and subsequent analysis of gas samples at GU by an unpredictably variable method of sample desorption.

Progress after the trials at SBEU led to the development of a more suitable means of desorbing samples collected on adsorbent resin, but unfortunately was too late for employment in the aforementioned trials.

Gas chromatographic analysis of gas samples by direct injection of headspace samples collected using gas-tight syringes was straightforward and daily practice at GU. Although as mentioned in chapter 2, the GC had to be fully calibrated and a graph produced every day it was in use, because of between day variability, the fluctuations within a day were negligible and consistent results could be easily attained. The maximum volume of gas

injected was 2ml, and this was quite often the volume used to permit low concentrations to be quantified using the GC.

3.1.1.1 Method

Two 5l dilution vessels were used to spike with incremental concentrations of ethylene from standard pressurised gas cylinders. Following injections of ethylene the vessels were left to equilibrate for fifteen minutes before sampling. Each vessel was completely air-tight and could be sampled via syringe through the septum on the top or a larger quantity of air could be drawn out of the vessel using the specially fitted side arms.

Originally the dilution vessels were round bottom flasks with only the one opening (circular ground glass type on a short neck). A septum port was added to this opening by extending the neck and making grooves at the top of the neck to allow the septum cap to be opened and closed for replacing the septum. The side arms were added as illustrated in the figure below, *Figure 5*. The joints in the side arms are made of Teflon. All the joints were greaseless to prevent interference with the GC traces.

Figure 5 5l dilution vessel used for determining the concentration of ethylene using both methods

Once the spiked concentration had been allowed to equilibrate a headspace sample of an appropriate volume was withdrawn via the septum port on the top of each vessel. This sample was injected directly into the GC for analysis and the concentration determined by comparison with a standard graph prepared earlier in the day.

After GC samples were taken one side arm was opened and 400ml (at 50ml/minute) was withdrawn using the Gastec system and a small piece of silicon tubing to connect the colorimetric tube to the side arm. There were two replicates of each concentration level for both Gastec and GC. The concentrations used are detailed in the following table, *Table32.*

Spike concentrations ppm				
0.0	0.2	5.0	10.0	20.0

Table 3 Concentrations of ethylene spikes used in correlating methods of determination

Here the 0.0ppm means that no ethylene was added to the dilution vessel. This was intended to provide a background level if at all detectable that could be subtracted from the other results.

3.1.1.2 Results and Discussion

The results of each vessel are presented separately because the spike level in the vessels was believed to be slightly different. This was reflected in both the GC and Gastec results, which corresponded well for each vessel. The actual concentrations and observations noted are given in the following tables.

First Vessel

Ethylene Concentration ppm		
spike	**GC**	**Gastec**
0.00	bld	bld
0.20	0.22	0.2
5.00	4.24	5.0
10.00	7.63	10.0-30.0
20.00	19.93	30.0

Bld = below the limit of detection

Table 4 Determination of ethylene concentration by GC and Gastec (vessel 1)

There was no background level detected using either method of determination.

At the lower end of the scale the two methods seem to correspond rather well providing the guidelines for readings from the Gastec colorimetric tubes are followed. The observations concerning how the readings were arrived at are stated in the following table.

The results are more congruent in the lower ranges because here the Gastec system is more reliable in providing a more closely focused result range, see *Figure 6* which displays that divisions on the scale are narrower at the lower end.

Figure 6 Scale divisions on a Gastec tube as used in the experiment

spike	Observations on gastec reading
0.00	no colour change
0.20	slight discolouration up to 0.2 mark
5.00	dark past 1 mark, paler towards 5 division
10.00	dark almost up to 10, pale far beyond 10, within 10-30
20.00	dark up to between 10-30, paler towards 30

Table 5 Observations on the Gastec readings from Vessel 1

Figure 7. is a graphical representation of the relationship between both methods. A major confinement on the use of such a graph in this circumstance is that on occasion only a range of concentration, as opposed to a definitive value, could be stated for determination by the Gastec system. For the purpose of the graph the mid-way point between 10-30ppm of 20ppm had to be used to allow plotting using Excel™ software.

The general relationship and how it changes as the ethylene concentration increases is clear. A linear trend is apparent over the lower end of the concentration range, but the relationship appears to become poorer and curved at the upper end of the concentration range. Here the Gastec system leads to overestimation of ethylene in the vessel by comparison with GC analysis.

For samples that are expected to be in the upper end of the overall concentration range GC would be the required method. It is the preferred method because it can be used to quantify with more precision and to lower concentrations.

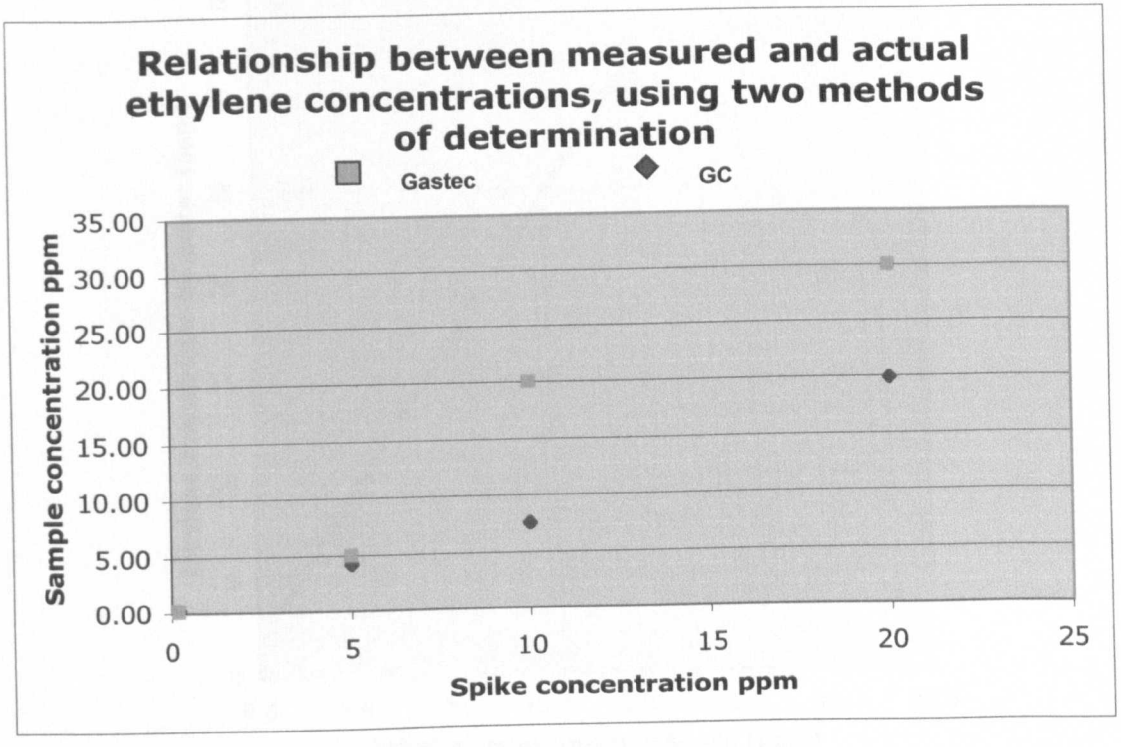


Figure 7 Graph of the relationship between measured and actual ethylene concentrations using two methods of determination (vessel 1)

The graph overleaf has the ethylene concentrations as determined by each method plotted against one another on a one to one scale with the r^2 value given for the linear correlation between the two methods. GC results are placed on the x-axis as they are considered more reliable and are the standard method here at GU.

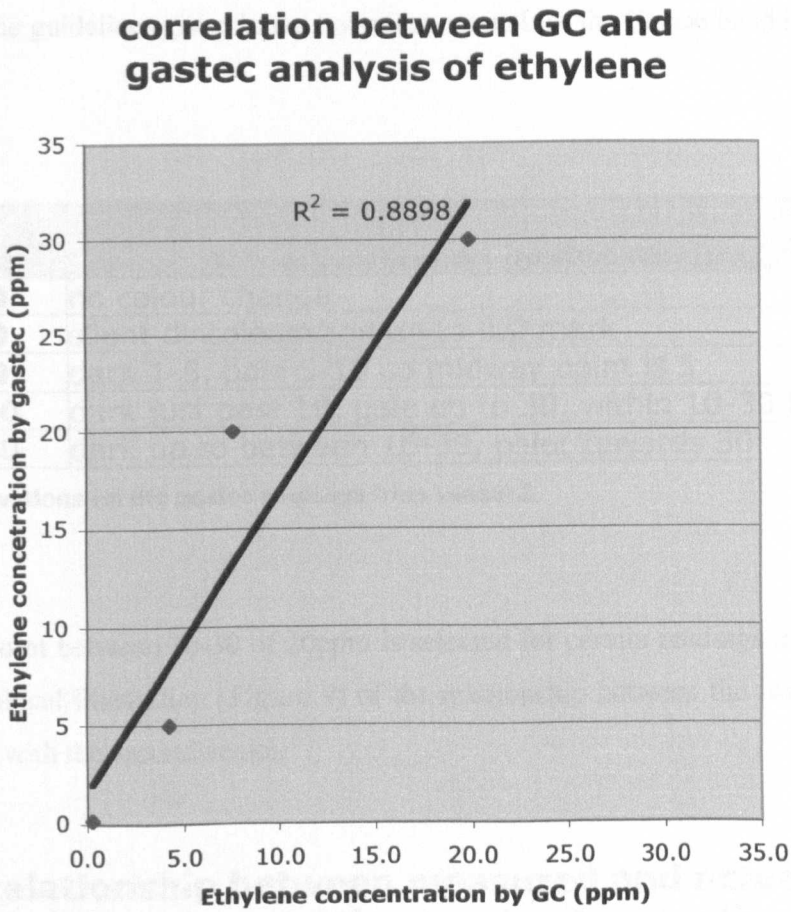


Figure 8 Graph of the correlation between ethylene determination by two methods (vessel 1)

Second vessel

Ethylene Concentration ppm		
spike	**GC**	**Gastec**
0.00	bld	bld
0.20	0.25	0.2
5.00	5.14	5.0
10.00	11.26	10.0-30.0
20.00	21.12	30.0

Table 6 Determination of ethylene concentration by GC and Gastec (vessel 2)

Again no background ethylene was detected by either method of analysis.

As before the methods relate well at the lower end of the scale, but drift apart as the Gastec system leads to artificially high valuations of the concentration present.

The observations associated with reading the Gastec results are given in the table below. They follow the guidelines given in the operation manual for the Gastec hand held pump.

spike	Observations on gastec reading
0.00	no colour change
0.20	slight discolouration up to 0.2 mark
5.00	dark 1-5, pale 5-10 so midway point is 5
10.00	dark just past 10, pale up to 30, within 10-30 bracket
20.00	dark up to between 10-30, paler towards 30

Table 7 Observations on the gastec readings from vessel 2

A mid-way point between 10-30 of 20ppm is selected for certain readings to produce the previous graphical illustration (*Figure 9*) of the relationship between the two methods of analysis used with the second vessel.

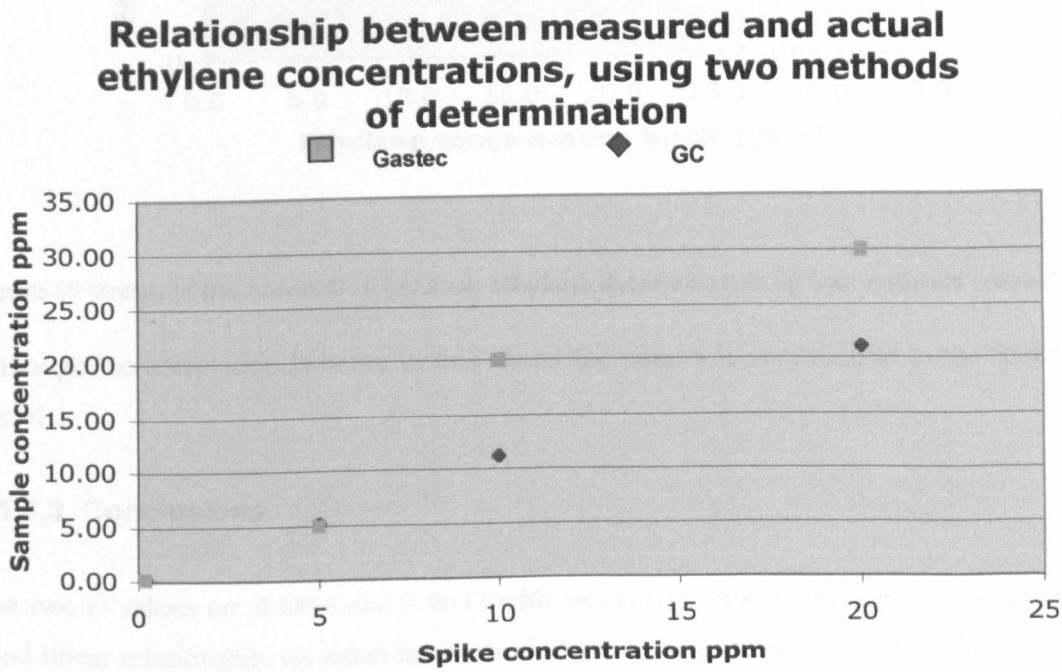


Figure 9 Graph of the relationship between measured and actual ethylene concentrations using two methods of analysis (vessel 2)

The relationship is very similar to that from the first vessel with the Gastec system consistently leading to higher results than GC analysis at relatively high concentrations.

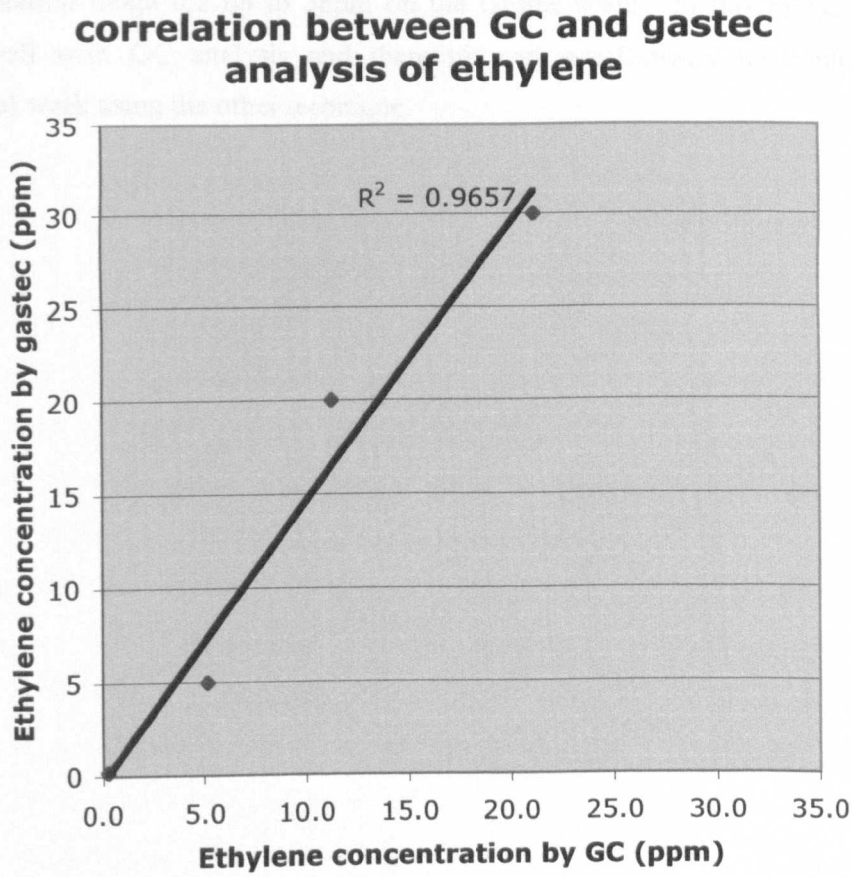


Figure 10 Graph of the correlation between ethylene determination by two methods (vessel 2)

Although the correlation is better in this vessel the pattern is very similar to the first test vessel.

3.1.1.3 Conclusions

The two r^2 values are 0.8898 and 0.9657 with an average of 0.9278, depicting an overall good linear relationship, although the methods digress more at higher values. The linearity is aided by the fact that the difference between the Gastec and GC readings at 10 and 20ppm spike concentrations is very similar. The conclusion that the relationship is a curve takes into account the fact that a mid-way point is selected from the 10-30ppm results and the actual value could be anywhere within this. Thereby ignoring this point and looking at the data a curve is clearly indicated, particularly in vessel one data set.

The exact point at which Gastec tubes begin to generate higher results is unknown, but based on these findings it appears to be somewhere between 5 and 10ppm. Fortunately the experiments in which Gastec was used throughout the project largely yielded levels within the concentration range 0.2 up to 5ppm on the Gastec scale. In this range the results correlate well with GC analysis and therefore can satisfactorily be compared with experimental work using the other technique.

3.1.2 Methods of assessing processing quality: Reducing sugar concentration compared with fry colour examination

After sprouting and disease control, processing quality is the ultimate concern to growers, store managers and processors. Therefore it is the benchmark by which all crop is assessed before it will be purchased. Maintaining high quality during storage is vital as it determines what a crop is worth at the point of leaving store. The reducing sugar concentration is the gauge used to quantify the processing quality of potatoes. These sugars cause darkening during the frying process, specifically glucose and fructose. The higher the concentration of reducing sugars the darker the resulting crisps or chips. Dark coloured products of the Maillard reaction are not aesthetically pleasing to customers and can influence the taste. As such they are not acceptable to the commercial market. Large companies like Walkers snack foods, MacDonalds, McCains and Golden Wonder have a representative sample of crop analysed for concentration of various sugars before purchasing goes ahead. Along with this, or in many cases instead of analysing sugar levels, the fry colour of a representative samples is assessed and decisions on shipping to the factory based around this. Basically the processing companies want to know what the processed product will look and taste like. Measuring sugars is an alternative to fry colour assessment, and is addresses the question of how much starting material is present for development of darkening, i.e. the pre-cursor to poor fry colour and what potential there is for darkening to occur. Hence these two methods are quite interchangeable and quite often those in industry can speak of either or both and know exactly what the significance is.

Of the reducing sugars glucose and fructose are key. When reference is made to reducing sugar levels it usually means the concentration of free fructose plus free glucose. Depending on the type of analysis done for reducing sugars, the other more minor soluble sugars are included. Typically HPLC separates these on the column in use for fructose and glucose, but it is not essential that they be included in the value examined as a marker for processing quality. On occasion sucrose values are desired to indicate the potential for further conversion to free reducing sugars.

Both fry colour and reducing sugar concentration methods have been used throughout this project, however only one type of analysis (either fry colour or reducing sugar content) was selected for each trial. The selection in each case was based on practicalities like where the trial was conducted, the equipment available at that time and what was the most suitable output type for the distribution and presentation of results as intended by the BPC.

The following experiment was designed to show the relationship between fry colour as processed and assessed per standard operating procedure and the reducing sugar concentration of the same tubers. Storage temperature was the factor used to create a shift in sugar balance and make the potatoes in different storage temperatures of various qualities so that the relationship between these two tuber attributes could be studied over different stages of processing quality.

3.1.2.1 Design and Method

Storage

Potatoes were held in storage at GU at comparatively cold, warm or optimum temperature conditions, of 4-6, 10-11, or 15-16°C. for a period of fourteen days. There were three replicates for each storage temperature. A replicate consisted of 6kg of tubers, cultivar Saturna in a 10kg box.

Sampling

On days 1, 7 and 14 of storage a representative sample (4/5 tubers) was taken from each box. The tubers were washed, peeled and halved longitudinally. Half was sliced and processed into crisps as per scaled down version of BPC standard operating procedure (*chapter 2).* Tuber numbers in the analysis were stepped down firstly, to ensure all samples could be completed by one individual and secondly, to be manageable in the smaller equipment. The other halves of the tubers from each replicate were diced, mixed and a representative sub sample of approximately 25g was extracted in methanol (~60ml). The extraction was conducted as described in the chapter 2.

Analysis

The crisp samples were kept in storage for a short period until taken to SBEU to use the facilities and established techniques of light box valuation and assignment of Hunter-L values for fry colour and defect analysis.

The extracts were analysed for reducing sugar content using Roe, Leloir-Roe and Somogyi-Nelson methods, which are detailed earlier in this thesis. This was conducted entirely at GU.

3.1.2.2 Results and Discussion

The mean of the three replicates from each storage temperature is used for comparing the methods. Error bars of plus and minus one standard deviation is given. Fry defects were also assessed but are not included as they are not directly related to the comparison being made. To begin with the result of the storage conditions are presented as resolved by each method of evaluation. Following this a comparison of methods is drawn.

The first graph shows the pattern of fry colours observed and how the storage temperature affected this parameter. A high Hunter-L value depicts a light crisp colour and hence good processing quality.

Fry colour

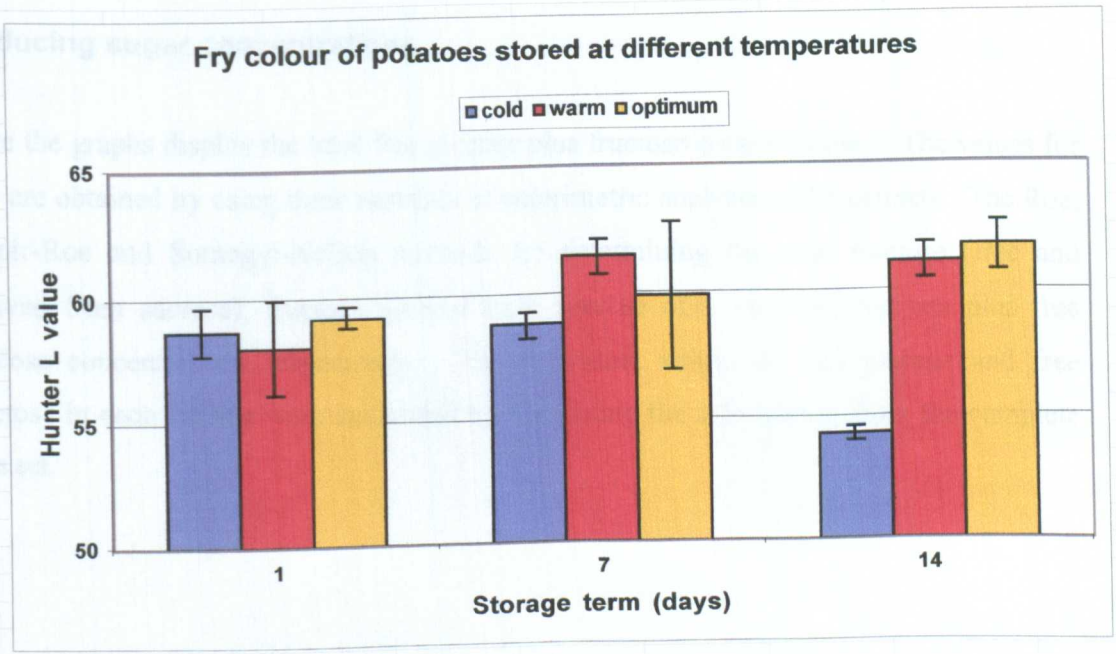


Figure 11 The effect of storage temperature on the fry colour of potatoes (n=3)

Treatment	Day 1	Day 7	Day 14
Cold	a	a	a
Optimum	a	b	b
Warm	a	ab	b

Table 8 Statistical analysis of L-values. Different letters denote significant differences

The effect of storage temperature after only one day was not very clear-cut. However after seven days of storage the results display quite plainly the significant difference between the two extremes of temperature. The error associated with the storage temperature considered to be optimum is larger and the effect cannot be significantly differentiated from either of the other two treatments. By day fourteen the normal pattern of poorer fry colour at low temperatures was well established. The cold treatment was significantly different to both the warm and optimum treatments on day 14. The effect of warmer than optimum storage was not significantly different to the optimum, even though it was expected to produce slightly lighter crisps because of the potential reconditioning and sugar burn off effect. The mean values imply that storage at this intermediate temperature would impart better quality. It is likely that no reconditioning occurred because of the late stage in the storage season that the trial was conducted, in May, when the sugar balance cannot be remedied easily.

Reducing sugar concentrations

Here the graphs display the total free glucose plus fructose concentrations. The values for this are obtained by using three methods of colorimetric analysis of the extracts. The Roe, Leloir-Roe and Somogyi-Nelson methods for determining the total fructose (free and derived from sucrose), fructose derived from sucrose only and free fructose plus free glucose concentrations respectively. The individual values of free glucose and free fructose in each sample were calculated by combining the information from the complete data set.

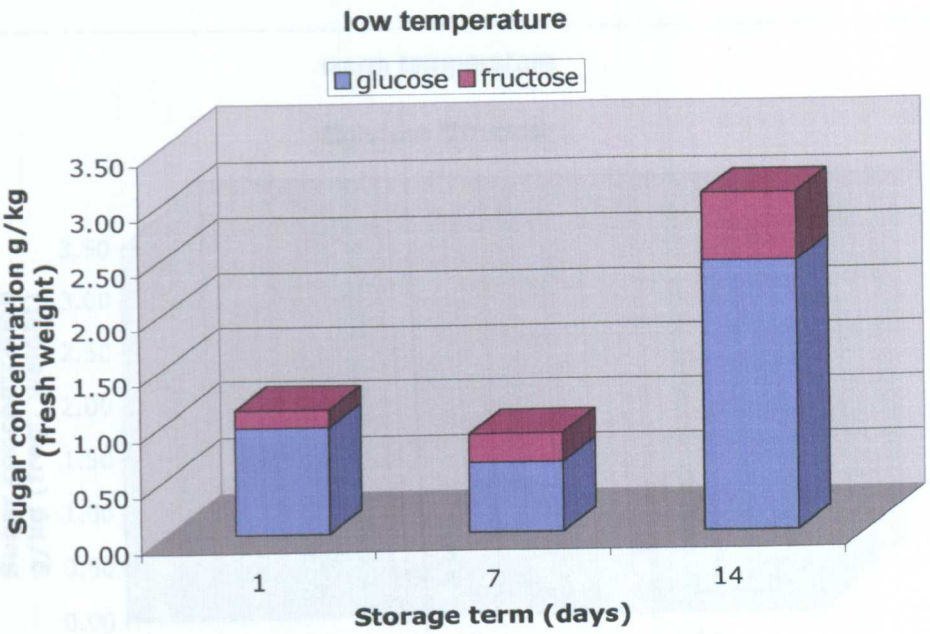


Figure 12 The effect of low temperature storage on reducing sugar levels over a short period

There was consistently more glucose than fructose present in all samples. Both sugars tended to follow the same pattern, with only one deviation from this seen above on day seven during cold storage. The fructose level increased albeit slightly from day one, but the glucose level dropped by a greater degree. Generally the negative effect of low temperature storage was evident by the sharp increase of reducing sugars by day fourteen to a significantly high level. This heightened starch conversion to sugar is typical of cold storage conditions.

The graph below showing the effect of relatively warm storage conditions illustrates how the distribution of sugars is affected when higher storage temperatures encourage growth of sprouts. Carbohydrates are essential energy for the growing sprouts and so are taken up and removed from the body of the tuber that will be assessed for quality. As sprout growth continues the effect will become more intense and the reducing sugar levels will remain low, only being replenished by the natural process of senescence. The main setback associated with warm storage conditions is outgrowth of sprouts, and correspondingly shrinkage due to loss of moisture. It is also important to note that warm storage promotes senescent sweetening. These are sufficient reasons for avoiding it entirely.

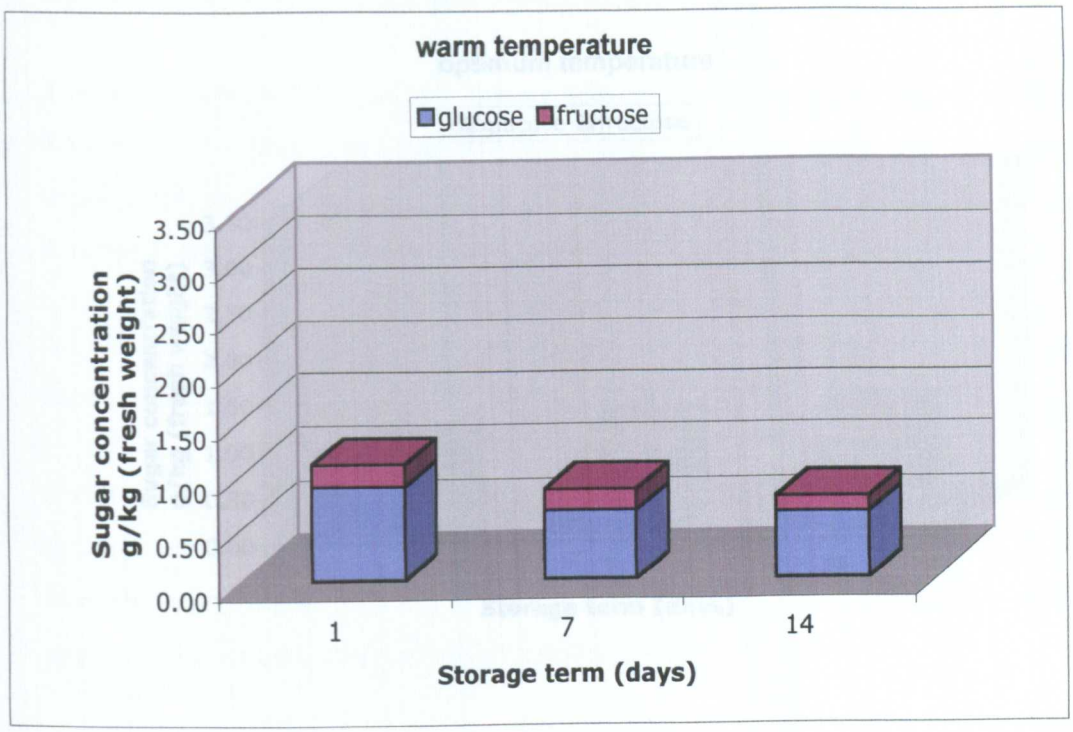


Figure 13 The effect of warm temperature storage on reducing sugar levels over a short time

The downward trend in reducing sugar concentration could suggest limited reconditioning. Perhaps the adjustment of sugars manifests itself more rapidly in reducing sugar concentrations than in development of dark colours on frying, so a small lag period may exist between these two features. If this were the case then any changes in the opposite direction would most probably be subject to an equal delay before the difference could be seen in fry colour.

The following graph is for the normal storage environment for cultivar Saturna and demonstrates the benefit of optimum storage conditions on reducing sugars. Over the course of fourteen days the fluctuation in concentration is quite small, similar to that from warm storage (although in the opposite direction). It is much less than the increase due to cold conditions. Both glucose and fructose increase over the storage term, but overall the changes are not extreme. The benefit is that this will not encourage sprouting or weight loss to the same degree as warmer temperatures.

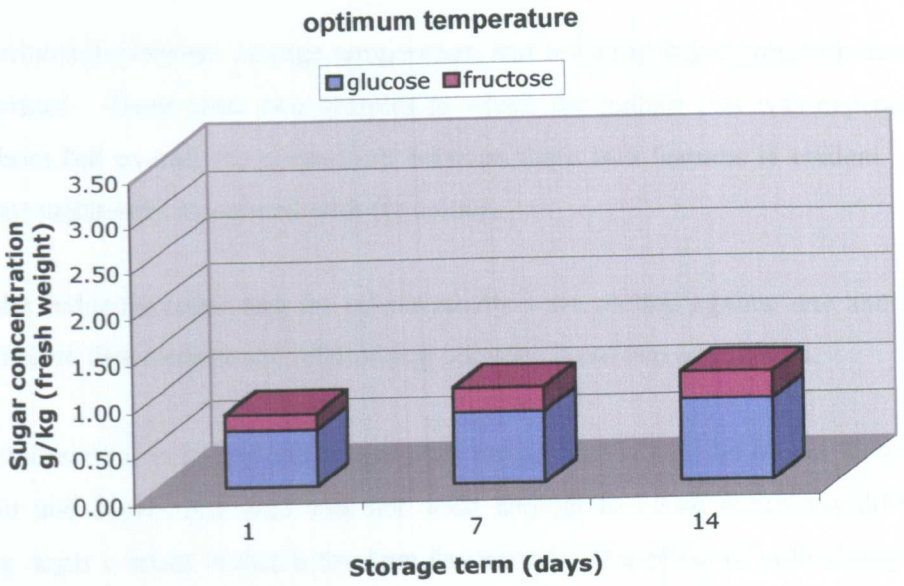


Figure 14 The effect of optimum storage temperature on reducing sugar levels over a short time

The reducing sugar levels are on the whole higher than desired for commercial acceptance of quality. It should be borne in mind that the trial was conducted in May at the tail end of the storage season when a fair amount of natural sweetening had already occurred.

Treatment	Day 1	Day 7	Day 14
Cold	a	a	a
Optimum	a	a	a
Warm	a	a	b

Table 9 Statistical analysis of glucose levels. Different letters denote significant differences

Treatment	Day 1	Day 7	Day 14
Cold	a	a	a
Optimum	a	a	a
Warm	a	a	b

Table 10 Statistical analysis of fructose levels. Different letters denote significant differences

Comparison of reducing sugar concentration and fry colour

The relationship between storage temperature and reducing sugar concentration has been demonstrated. There were two samples in which the pattern that was expected did not come about but overall the connection between these two features is evident. Reducing sugars are negatively associated with fry colour.

All of the reducing sugar and fry colour results were plotted against one another on the same graph to demonstrate the relationship between these two parameters.

The first observation is that the temperature range over which the tubers were stored for optimum and high conditions was not wide enough to cause enormous differences in reducing sugar content within a fourteen day period. The effect of cold storage had only begun to become evident after the full fourteen days.

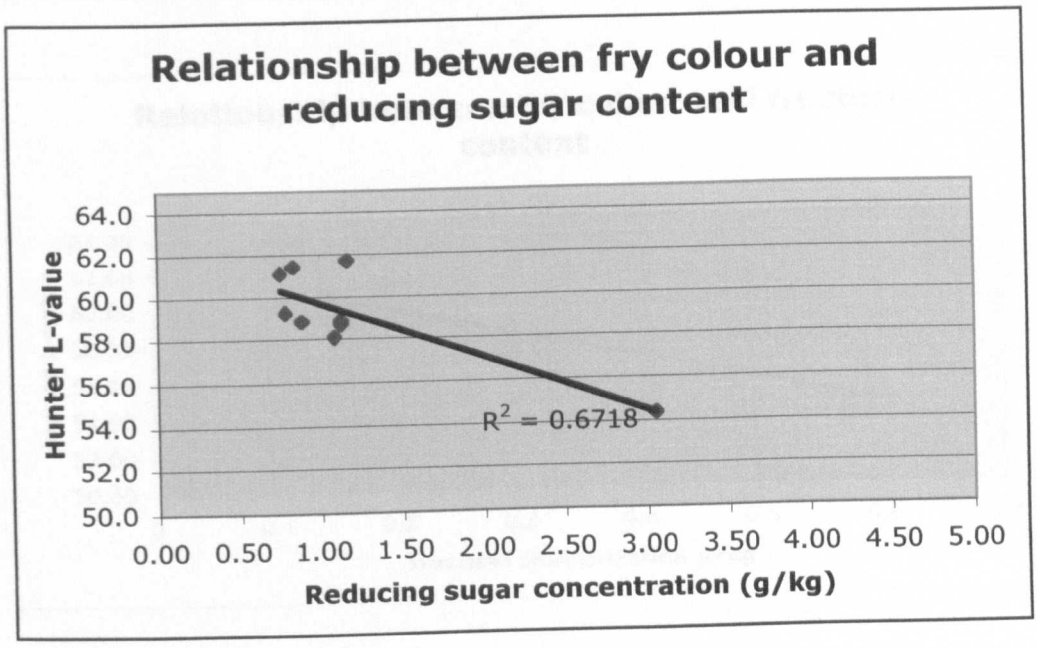


Figure 15 Graph of the relationship between reducing sugar content and fry colour

There does not appear to be a particularly good relationship (r^2 value of 0.6718) between fry colour and reducing sugar content from this graph, but roughly speaking it can be said that a concentration of approximately 0.5g/kg fructose plus glucose will yield an L-value of between 58 and 62. This corresponds to the industry standard (*Dr M. Kirkman, personal communication*). A change in L-value between these two levels is quite significant in terms of commercial acceptance of fry colour. It was expected that the trial conditions would have led to more extreme differences between both sugar levels and fry colour, but this was not the case. Longer storage time may have provided larger differences between samples. More sample numbers would have allowed the reproducibility of the pattern to be assessed and therefore determine whether any predictions of fry colour from reducing sugar results was possible.

Perhaps the timing of the trial and the corresponding physiological state of the tubers was not the best for creating big differences between treatments. Potatoes in storage react more to factors affecting sugar balance earlier in the season, but once senescent sweetening is underway, and in this case it is most likely, the reactions are less extreme.

When the sugars are plotted individually against the fry colour the relationship is better than with total reducing sugar values.

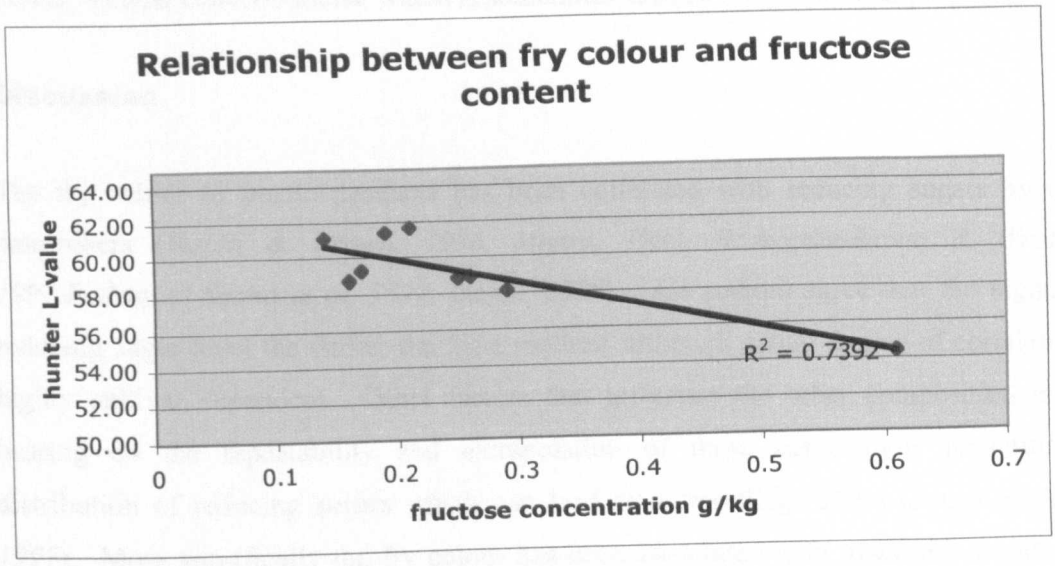


Figure 16 Graph of the relationship between fry colour and fructose content

The best correlation was found when L-values were plotted against fructose content, but this was still not good enough to be certain of the linearity.

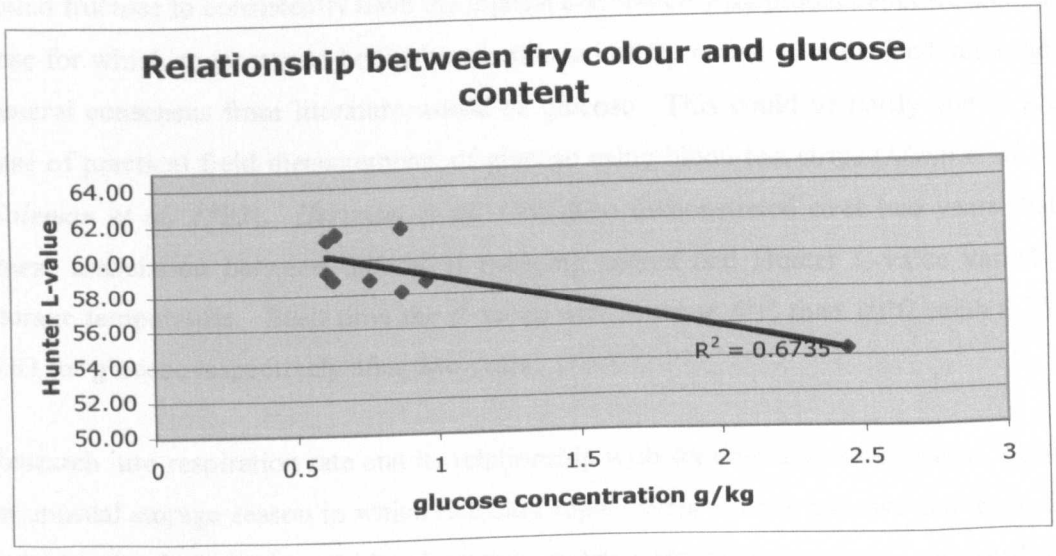


Figure 17 Graph of the relationship between fry colour and glucose content

This is only marginally better than plotting both sugars at once. It is clear from the x-axis in the previous graphs that the glucose and fructose concentrations are not present in the tubers in equal concentrations, which is sometimes assumed for analytical purposes.

Discussion

The fry colour of potato products has been correlated with reducing sugars by many researchers (*Habib & Brown, 1956, Mazza, 1983, Rodriguez-Saona & Wrolstad, 1997,Rodriguez-Saona et al, 1997, Uppal, 1999*). All authors agree that the higher the reducing sugar level the darker the fried product, although actual degree of correlation is highly cultivar dependent. Other factors that influence the tuber composition have a bearing on the repeatability and extrapolation of these associations including the distribution of reducing sugars which can lead to a 'mottling' effect (*Jankowski et al, 1997*). More specifically the fry colour has been associated with glucose concentration. An r^2 value of 0.89 was generated from results of fry colour measured by reflectance and glucose concentration (*Mann et al, 1991, Coleman & Tai, 1999*). Further to this *Pritchard & Adam, 1994* found that over the course of three years studies fry colour could be more closely associated with glucose than fructose or total reducing sugars, when potatoes were stored at 8°C. A reasonably high r^2 value of 0.80 was again shown for the glucose to chip (crisp) colour relationship by *Fick & Brook, 1999*. Alternatively *Herrman et al, 1996* found fructose to consistently have the highest correlation with product appearance. So the case for which sugar would be the best indication of fry colour is undecided, although the general consensus from literature would be glucose. This could be partly due to greater ease of practical field measurements of glucose using blood test strips (*Mann et al, 1991, Coleman et al, 1993*). *Herrman et al, 1996* also demonstrated over two years that the linear association between individual reducing sugars and Hunter L-value varied with storage temperature. Each time the r^2 value was better at 4°C than 10°C, with 0.75 and 0.63 for glucose respectively after two years.

Research into respiration rate and its relationship with fry colour was conducted following an unusual storage season in which reducing sugar levels did not increase above what was considered to be a detrimental level yet unacceptable product colours were observed (*Copp et al, 2000*). This work showed that reducing sugars could not always be reliably correlated with crisp appearance. There was evidence to suggest that respiration rate was related to fry colour but the correlation was very variable over the time scale studied.

A major conclusion for the purposes of this study, taking account of the foregoing discussion, is that the interpretation of results from both reducing sugar concentrations and fry colour of samples from different experiments within the project is a valid approach.

The chart on the following page is supplied by the BPC as a fry colour chart for crisping potatoes. It relates Hunter L-values to the PCSFA scores assigned subjectively to crisp samples. It is shown here purely to illustrate what the typical Hunter-L values mean in terms of actual crisp colour. Full details of the both methods of analysis are given in chapter 2.

Figure 18 Fry colour chart for crisping potatoes (*BPC, technology transfer*).

3.1.3 Analysis methods of reducing sugar concentration: Roe, Leloir-Roe and Somogyi-Nelson methods compared with High Performance Liquid Chromatography method

As detailed in the materials and methods section the chosen methods for sugar analysis at GU were that of the Roe, Leloir-Roe and Somogyi-Nelson. It was decided that it would be beneficial to compare a standard commercial analysis method with that employed at GU.

To accomplish this contact was made with Walkers Snack Foods Ltd. They kindly arranged to carry out HPLC analysis on samples already extracted at GU. The samples used were a combination of extracts from ethylene exposure experiments to hopefully cover a broad spectrum of concentrations arising from former treatments. The samples were extracted in methanol. A 2ml aliquot of each sample was placed into a septum capped glass vial. The vials were prepared for posting by strapping to cold packs from the freezer to keep them cool in transit. They were sent to Frito Lay analytical services laboratory in Leicester.

Unfortunately there were a number of problems with running the samples at Frito Lay. Finally when separation by HPLC was possible the traces of the samples completed were acceptable as an indication of the sugar levels but in agreement with the analyst they could not be used to assess method correlations. Further samples were sent to Frito Lay but time was not available to analyse them.

Forgoing the comparison of sugar methods, the relationship between the methods of ethylene analysis and that of fry colour and reducing sugar levels are satisfactory for comparison of results.

4 Chapter Four

4.1 ASSESSMENTS OF THERMAL FOGGING MACHINES

This chapter covers the more practical and applied side of the project aims. Investigation of the thermal fogging process was essential for assessing the fundamental features of the system such a temperature and flow rate and the how these interact with the potatoes in store. There is a lack of academic literature covering this technical area and therefore many of these studies are based around the thoughts and recommendations of the machine operators.

4.1.1 The thermal fogging process

Thermal fogging has long been considered to be the most effective method of CIPC application in potato stores. Initially several small fogger machines were positioned around the perimeter of stores and typically there would be six ports through which the CIPC formulation was fogged. This approach provided good distribution of the fog within the store and hence effective sprout control from the first application. Little was known about the particle size of CIPC produced by these smaller machines, however it is generally accepted that they produce smaller particles than the larger fogging machines.

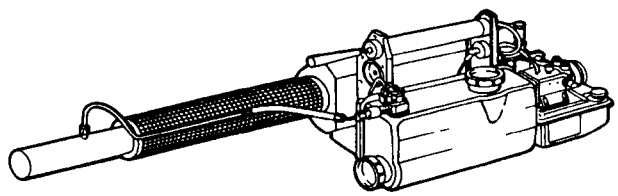


Figure 19 Diagram of the Swingfog machine formerly used in common practice for CIPC application in stores

The principles of fog generation utilized by the Swingfog are based on a pulse jet system. A series of fuel and air pulses are mixed and fired in the specially shaped combustion chamber. The resulting detonation creates a column of gas, which pulsates at approximately 90 times per second. The CIPC solution is delivered into the pulsating gas stream at the exhaust end, volatilized into billions of small particles and discharged. This

produces a dense fog which is directed into the potato store. At this point the temperature of the fog should be in the region of 40 to 60°C.

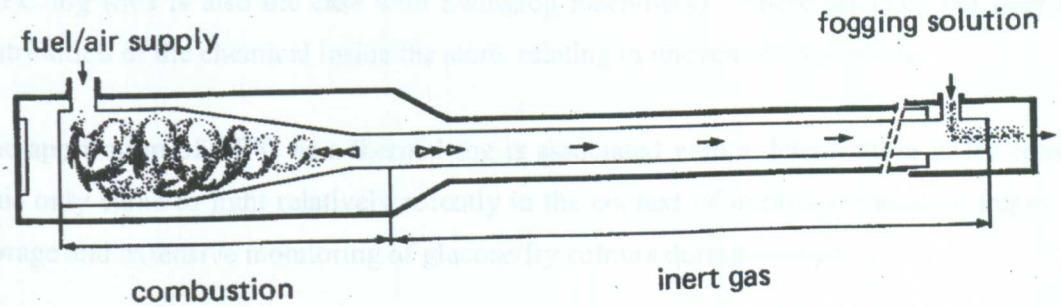


Figure 20 Schematic diagram of the working principles of the Swingfog machine

The drawback with this process was the length of time required for application. The flow rate of the CIPC formulation through the fogger was reasonably slow at 20l/hour. Over the years the machinery involved in the fogging process has been developed and new technology integrated with the basic system. Presently the standard commercial machines used for CIPC application in the UK are more powerful and have a high capacity of air and fog throughput. This allows application to be conducted over a considerably shorter time period (e.g. one hour to treat a 2000 tonne box store).

Figure 21 Representative commercial fogger machine in use currently in the UK (SCC, Unifog)

The advantage of the improved efficiency is greater convenience for both applicators and store managers. Other positive modifications include greater control of CIPC particle size (in the region of 3μm diameter) fuel delivery rate and hence application temperature. The associated disadvantages include exhausting combustion gases into store along with the CIPC fog (this is also the case with Swingfog machinery). There are concerns over the distribution of the chemical inside the store, relating to uneven residue levels.

The application of CIPC as a thermal fog is associated with a deterioration in fry colour. This only came to light relatively recently in the context of improved varieties, improved storage and extensive monitoring of glucose/fry colours during storage.

The introduction of a hot fog into potato stores has a disruptive influence. It can physiologically alter the potatoes by creating a stressful environment. Tuber respiration rate increases and so the crop will age. Initially the fry colour problems were linked with carbon dioxide in combustion gases and from increased respiration. When the problem arose, the more enlightened store managers linked it with CO_2 accumulations, due mainly to the extended interval (up to 24 hours) between application and ventilating the stores. No-one in industry (in the UK) was aware of the ethylene connection (*Dr. M. Kirkman, personal communication*).

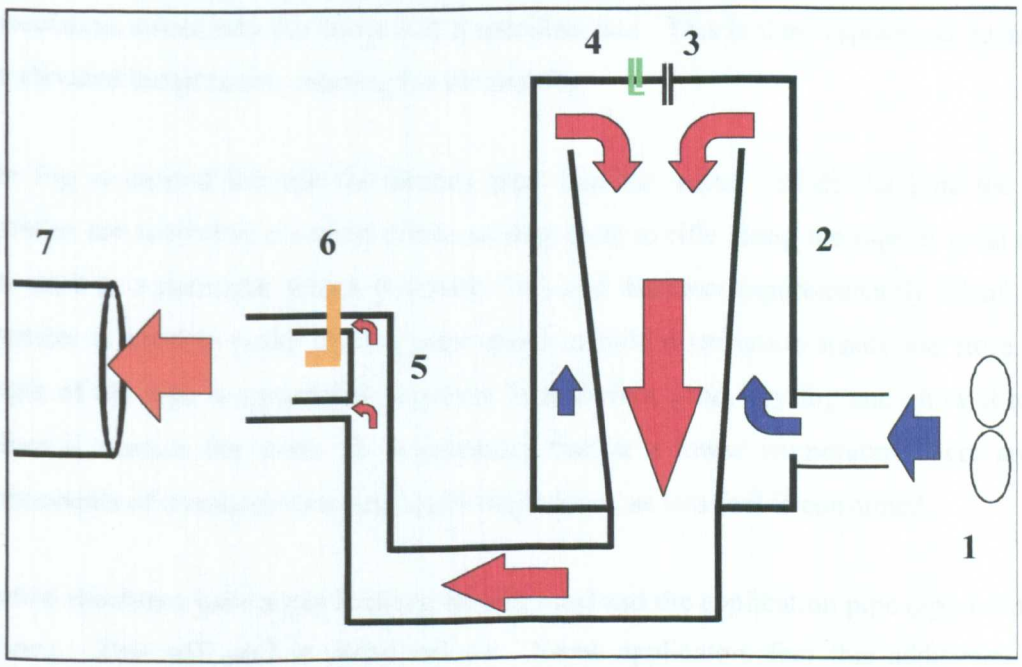
The increased respiration rate leads to elevated levels of carbon dioxide at times of restricted ventilation. There is evidence that when carbon dioxide concentrations are high crop will decline in processing quality (*Mazza & Siemens, 1990, Briddon & Jina, 1999*). Sprout growth and weight loss will also be affected. The outcome of exposure to elevated carbon dioxide will be influenced by the duration of exposure. However ambient levels will not adversely affect the processing quality of potatoes in storage.

Studies revealed that ethylene was produced naturally by crop (*Breech et al, 1973, Burton, 1989*) and from the combustion of petrol used to generate thermal fogs (*Wang & Pritchard, 1997*). Ethylene is present in thermal fogs as a by-product of burning the hydrocarbon fuel used to generate the fog. The ethylene created in a standard application is sufficient to induce a physiological response in tubers. Exposure of crop to ethylene effects reducing sugar concentration and hence fry colour (*Prange et al, 1998*). The extent of the outcome depends on exposure time and concentration.

Representative commercial fogging machines were investigated to provide a greater working knowledge and understanding of the process. The aim was to determine what

feature(s) of the system are causing the characteristic sweetening of tubers that occurs after hot fog treatment.

The following schematic diagram (*Fig 22)* illustrates in elementary terms how thermal fogs are created using these machines (*B Cotter, & A. Briddon, BPC, personal communication*). In summary, the CIPC is introduced to the hot air stream created by the machine. This generates a fog, which is carried into the store by a flexible ducting pipe. The outlet of this pipe is positioned in store for optimum distribution.



1. Blower pulling in ambient air
2. Combustion chamber
3. Fuel injection nozzle
4. Ignition
5. Hot air stream into fog head
6. CIPC formulation delivery nozzle
7. Application pipe to store

Figure 22 Schematic diagram of the thermal fogging process

The blower pulls in ambient air to control the volume and speed of air required for fog. The combustion chamber is where the fuel is burned and the cold air is heated to the appropriate temperature. Commonly most commercial operators use lead replacement fuel, because it burns at a lower temperature than unleaded petrol therefore causing less wear on parts. An electric pump controls the rate of fuel delivery. This is adjustable using the pressure relief valve. As the pressure is altered, correspondingly so is the fuel consumption rate and hence the temperature respectively. As the pressure is reduced less fuel is supplied and the burner temperature lowers.

Following fuel injection and ignition, burning the petrol creates energy; it is this energy that heats the cold air stream. Also produced by burning hydrocarbon fuels are the combustion gases mainly carbon dioxide and water vapour. However as no mechanical system is 100% efficient there will be some unburned fuel and products of incomplete combustion i.e. contamination (e.g. benzene, polyaromatic hydrocarbons and ethylene).

Typical application temperatures range from 330°C to 500°C depending on the applicator's preferred conditions. The burner temperature that the fogger is set to operate at is a measure of the air temperature immediately before the flame trap (the hottest point).

The hot air stream (including exhaust gases) is delivered into the fog head and the CIPC formulation enters into this airflow at a specified rate. This is then vapourised because of the elevated temperature, creating the thermal fog.

The fog is carried through the ducting pipe into the store. Inside the pipe the CIPC particles are subject to a venturi effect causing them to rifle along the pipe at great speed. The pipe is a particular length (typically 7m) and diameter (approximately 10cm). The diameter is used to make the fog gain speed to aide distribution inside the store. The length of the pipe is considered important to achieve a good, dry fog and allow it to cool before it reaches the store. It is estimated that at a lower temperature there are less components of a contaminant nature entering a store, as less fuel is consumed.

Certain machines have a gap between the fog head and the application pipe (approximately 2.5cm). This will pull in additional air. Some applicators feel this additional air is required to carry all the fog into store and move it around sufficiently once inside the store (*N. Green, personal communication*).

In addition to what is depicted in *Figure 22*, there is a control panel, where the running conditions can be managed and monitored throughout application (*Fig 23).*

Figure 23 Control panel on commercial fogging machine used for controlling application conditions

4.1.2 Assessment of the gases produced by thermal fogging machines in a commercial store

The objective was to assess the levels of C_2H_4 and CO_2 present in a commercial store as a result of thermal fogging. 750 tonne bulk stores in Cayton, Scarborough (M^CCain) were treated with a thermal fog application of a CIPC formulation.

Figure 24. below illustrates the layout of the stores at the site. The grey panels mark the passage tunnels. The tunnels have a door at the mid-way point between each set of stores i.e. between set 2 and 4 and set 1 and 3. Stores 2 and 4 were treated with the same application of MSS 50M, a methanol-based formulation. The roof covering is communal to stores 1, 2, 3 and 4, however the walls of the passage tunnel partitions the roof space into two separate sections. There was also a partial divide in the air space between each set of stores (1 & 2 and 3 & 4). The partition from the roof reaches down to approximately 0.5m above the crop and runs width ways between the passage tunnel and the side of each store. There was an identical roof and air space arrangement in stores 5, 6, 7 and 8. Stores six and eight were treated with the same application of Warefog, a methylpyrrolidone-based formulation. The headspace above the potatoes in a full store was approximately 1.5m.

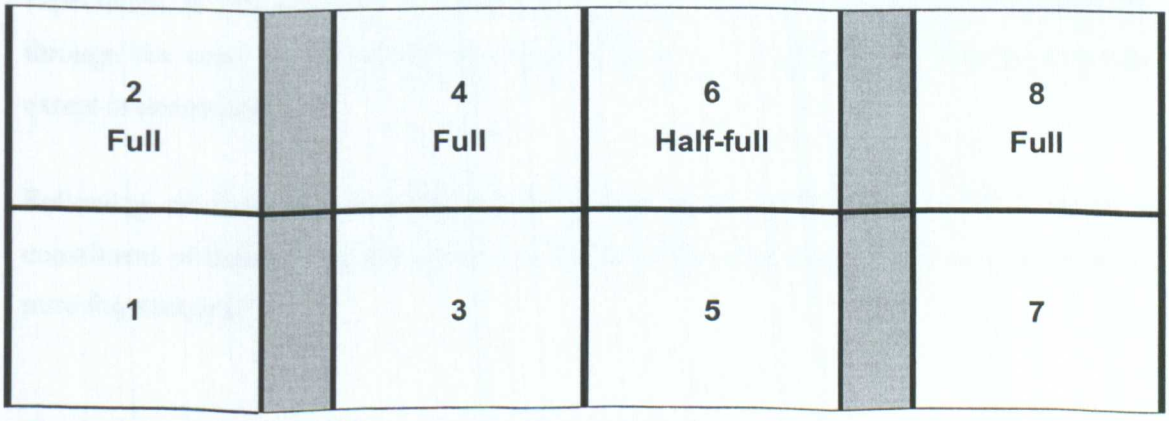


Figure 24 Layout of the M^CCain stores at Cayton, Scarborough

These bulk stores had cylindrical shaped lateral ducts. In each case the fog was applied into the passage tunnel between the stores. The pressure of the fog forces it to travel through the lateral ducts and up through the potatoes themselves.

Applications were conducted by an experienced applicator (Nick Green, SCC) using a Unifog machine at burner temperature 475°C. The flow rate of CIPC formulation into the

hot air stream was 1l/min and 0.8l/min for MSS 50M and Warefog respectively. MSS 50M was applied at a dose rate of 28.5ml/tonne and Warefog at a dose rate of 25ml/tonne.

The crop in all stores was Russet Burbank. Crop temperature was maintained at 10°C.

CO_2 and C_2H_4 measurements were taken from headspace and lateral ducts, positioned mid-way along the passage tunnel. These areas were sampled prior to the applications and 24 hours after completion of application. No further samples were collected after ventilation.

C_2H_4 was not detected using the semi-quantitative method employed in this experiment (Gastec colorimetric tubes, see chapter 2). This method is sensitive to 0.2ppm C_2H_4 in air. CO_2 levels were overall no higher following application than those detected before thermal fogging. The maximum concentration was in store 2, 24 hours after application at 0.3% CO_2.

The relatively low CO_2 levels were surprising as up to this point they had been considered the reason for darker fry colours after fogging. These unexpected results suggested that there was significant air exchange between the store and ambient air in the 24-hour period between application and sample collection. This air exchange had flushed the headspace within the store of any detectable C_2H_4 that may have been introduced by fogging. The expectation is that normally a certain percentage of the fog will be lost to outside air through the vents on the roof of the stores. However unintentional air exchange to this extent is uncommon.

Following on from this the question was raised as to whether ethylene was indeed a constituent of thermal fog and could it be identified in storage atmospheres as opposed to pure fog samples?

4.1.3 Assessment to determine whether Ethylene is produced by the thermal fogging process

Trials were conducted in 12 tonne stores at SBEU. The work was undertaken to determine whether C_2H_4 could be detected in appreciable amounts in store atmospheres as a result of thermal fogging. There was no crop present in the stores. The stores were completely sealed simultaneously as the CIPC fog applications ended. This prevented any build up of excess pressure and ensured no leakage or exchange with ambient air for the 24-hour period following application.

CIPC applications were conducted using a Swingfog machine, as it was not necessary to use an industrial fogger for the purposes of the experiment. The Swingfog works on the same principles as the larger commercial fogger machines, but it is not as powerful. Therefore it should only be used to distribute fog in smaller areas. It is ideal for use in the 12 tonne stores. One store was fogged with Luxan Gro-stop, a dichloromethane-based formulation at a rate of 60ml/tonne. The second store was fogged with MSS 50M formulation at a rate of 42.5ml/tonne. The dose applied was based on a theoretical six tonne load of crop in stores. The dose rates used were those most often adhered to for the first CIPC application during the storage season. 360ml of Gro-stop was applied and 255ml of MSS 50M.

The flow rate of the formulations through the Swingfog was significantly different due to the viscosity of the CIPC solution (methanol is less viscous than dichloromethane therefore it can flow through the delivery nozzle faster at ~0.5l/min compared with ~0.3l/min respectively). The time taken to apply the Gro-stop was much greater than the time taken to apply MSS 50M. Therefore exhaust gases from the combustion of hydrocarbon fuel source were entering the store treated with Gro-stop for a longer period of time.

Headspace samples were collected 5 and 24 hours after fog application, prior to ventilation (C_2H_4 by Gastec). In both stores C_2H_4 was present at both 5 and 24 hours after fogging. The concentration in the store fogged for the longer period (Gro-stop) had the higher C_2H_4 concentration of 30ppm 5 hours after application and 10-30ppm 25 hours after compared with 5ppm at 5 hours and 1-5ppm 24 hours after the shorter MSS 50M application.

When air exchange is controlled to prevent leakage into the surroundings even a volatile compound such as C_2H_4 can remain in the store atmosphere for the full 24 hours prior to ventilation.

4.1.4 Assessment of representative commercial thermal fogging machines

Having determined that C_2H_4 is produced by fogging equipment (Swingfog) and enters potato stores with the exhaust gases, an assessment of representative commercial fogger machines was conducted. The study was undertaken at SBEU and encompassed a range of different fogging systems and CIPC formulations currently in use for sprout suppression throughout the UK. The aim of the experiment was to determine levels of C_2H_4 and CO_2 present in store atmospheres as a result of thermal fog applications.

Three applicators took part in the study: SAM (Unifog machine), Stored Crop Conservation (SCC) (Unifog machine) and Superfog Ltd (Superfog machine). Each applicator used their own equipment and applied two formulations, one of which was common to all three of them (MSS 50M). On each occasion the formulation was applied at the operator's optimum conditions[1] then conditions selected by myself. Where possible application temperatures lower than the optimum were selected for the second set of conditions. In the case of the SAM unifog applying Gro-stop the second temperature had to be higher as a good quality fog could not be guaranteed any lower than at the optimum temperature. The expectation was that at lower burner temperatures less C_2H_4, CO_2 and general contaminants would be produced. At a lower burner temperature less fuel is consumed. Lastly the fogger machines were run at the operator's optimum conditions without any CIPC formulation introduced; thus only the exhaust gases entered the store. The table below (*Table 11*) indicates which machinery was used to apply each CIPC formulation.

Fogger Used	Formulation Applied	
SAM, Unifog	MSS 50M	Gro-stop
Stored Crop Conservation, Unifog	MSS 50M	Warefog
Superfog Ltd, Superfog	MSS 50M	MSS 50M

Table 11 Design of fogger machine assessment trial

[1] The SAM Unifog machine involved did not have optimum conditions for application of MSS 50M, therefore running conditions based on the optimum for MSS 50M use with SCC Unifog were used.

The variables compared were fogger machine, formulation applied and application temperature.

Applications were conducted into empty twelve tonne stores to ensure that no C_2H_4 measured had emanated from potatoes (potatoes are known to emit C_2H_4 particularly when subject to stressful conditions for example elevated store temperature during application).

Stores were maintained at 10°C prior to the start of fogging. Each store was fogged with a dose of CIPC tailored for a theoretical load of twelve tonnes. The CIPC formulations were applied at rates commonly used for the first treatment of the storage season. The dose rates used were 42.5ml/tonne for MSS 50M, 25ml/tonne for Warefog and 60ml/tonne for Gro-stop.

The flow rate of formulations through the fogger machines was different, depending on the formulation delivery nozzle and the solvent used. The duration of each application was calculated beforehand using this information. The SAM Unifog delivered each formulation into the hot air stream at a rate of 0.98l/min. The Superfog machine delivery rate was 1l/min. The SCC Unifog delivered the methanol-based formulation (MSS 50M) at a rate of 1l/min and the methylpyrrolidone-based formulation at a rate of 0.8l/min.

Each applicator carried out six treatments. The treatments conducted are outlined below in *Table 12*.

SAM, Unifog						
Formulation	**MSS 50M**	**MSS 50M**	**Exhaust**	**Gro-stop**	**Gro-stop**	**Exhaust**
Application temperature	400	470	470	400	330	330
Temperature conditions	lower	optimum	optimum	higher	optimum	optimum
Stored Crop Conservation, Unifog						
Formulation	**Warefog**	**Warefog**	**Exhaust**	**MSS 50M**	**MSS 50M**	**Exhaust**
Application temperature	500	400	500	400	500	500
Temperature conditions	optimum	lower	optimum	lower	optimum	optimum
Superfog Ltd, Superfog						
Formulation	**MSS 50M**	**MSS 50M**	**Exhaust**	**MSS 50M**	**MSS 50M**	**Exhaust**
Application temperature	350	450	450	350	450	450
Temperature conditions	lower	optimum	optimum	lower	optimum	optimum

Table 12 Details of the treatments conducted for assessment of representative thermal fogging machines (application temperatures in degrees Celsius)

Stores were sampled for headspace concentrations of C_2H_4 and CO_2 (C_2H_4 by Gastec, CO_2 by HiTech landfill gas meter) over a twenty-four hour period (before ventilation). Samples were collected prior to CIPC applications, 4-6 hours after and approximately 24 hours after treatment. The HiTech Instruments gas landfill meter used for measurement of CO_2 is sensitive down to 0.1% CO_2 in air.

CO_2 levels in all stores that received treatments from the SAM Unifog machine had undetectable levels of CO_2 on all three sampling occasions. In the stores treated with SCC Unifog and Superfog machines the highest concentration detected in the store atmospheres was 0.1% CO_2.

Even in these restricted air exchange conditions the CO_2 introduced to potato stores by this range of typical thermal fog applications was not sufficient to cause the darkening of fry colour. Particularly as it is common practice for all stores (experimental and commercial) to be vented 24 hours after application. The maximum build up of CO_2 projected in a commercial store from these trials would be 0.1% under similar conditions.

C_2H_4 concentrations generally did not exceed 5ppm and in many cases only reached a maximum of 1ppm. The exact concentrations in each store following treatment are in the table below (*Tables 13*).

Ethylene concentration in store atmospheres (ppm)								
SAM, Unifog			SCC, Unifog			Superfog Ltd, Superfog		
Sample time (hours post-fog treatment)								
Treatment	6.5 h	28 h	Treatment	4.5 h	22 h	Treatment	4.5 h	27 h
MSS 50M 400	5	1	MSS 50M 400	1	1	MSS 50M 350	1	1
MSS 50M 470	5	5	MSS 50M 500	5	5	MSS 50M 450	1	1
Exhaust 470	30	10	Exhaust 500	5	1	Exhaust 450	1	1
Gro-stop 330	1	1	Warefog 400	1	1	MSS 50M 350	1	1
Gro-stop 400	1	1	Warefog 500	5	5	MSS 50M 450	5	5
Exhaust 330	1	1	Exhaust 500	5	1	Exhaust 450	1	1

Table 13 Ethylene concentrations in store atmospheres after thermal fog application (burner temperatures in degrees celcius)

Excluding the SAM, Unifog Exhaust 470°C treatment, generally there was a modest but not significant difference in the C_2H_4 produced by different thermal fogging machines or CIPC formulations used. Generally certain machines appear to be more efficient at burning petrol under similar conditions as differing amounts of C_2H_4 are formed.

When exhaust only is fogged into stores, C_2H_4 is produced in equivalent amounts to when CIPC formulations were applied. As originally anticipated the C_2H_4 generated by thermal fogging is a product of the application process and not a function of the active ingredient (CIPC) or the solvent used in the formulation.

The running conditions of the fogging machines did impact on the amount of C_2H_4 produced. Overall as more fuel was burned to run at higher temperatures an increased amount of C_2H_4 was detected in store atmospheres. This pattern was consistent in all the stores treated with the SCC Unifog machine.

The SAM Unifog results clearly indicate the machine was optimized for application of Gro-stop. The relatively low application temperature is well suited for the formulation and is practical for reducing C_2H_4 production. When the machine was used for application of a second formulation with a different solvent base, it did not perform as well. The burner temperature was higher for the MSS 50M (as is normal for application of this formulation) and as such more C_2H_4 was expected. However when the exhaust only from this machine was ducted into store the C_2H_4 concentration determined was far in excess of that of any other sample. This is a good example of how machines can be well developed for a specific use, in this case application of Gro-stop. Yet when parameters are altered without the required time spent on optimization the overall performance of the equipment can vastly deteriorate.

The volatile C_2H_4 found to be present in store atmospheres following fogging was detectable for the full 24 hours preceding ventilation. The persistence of this compound was surprising considering its volatile nature.

The concentration and the duration of exposure time of potatoes to C_2H_4 were identified as areas of immediate concern, based on the knowledge of C_2H_4 and its potential impact on dormant tubers.

4.1.5 Secondary trials with representative commercial thermal fogging machines

The aim was to verify that the application process produces C_2H_4 under normal operating conditions when potatoes are in store.

The stores used in this trial had a smaller capacity than those previously tested for C_2H_4 concentration following thermal fog application. These six tonne stores at SBEU had a full load of crop.

The crop temperature was maintained at 10°C prior to applications and again when store systems were switched on approximately twenty-four hours after application. All fog applications were conducted using a Superfog Ltd, Superfog machine, by a qualified applicator (David Wagstaffe, Superfog Ltd).

The flow rate of the MSS 50M formulation through the machine was 1l/min. The dose of CIPC applied and therefore the duration of fogging was calculated based on the six tonnes of potatoes present. The run time was fifteen seconds for each treatment. The treatments are outlined in the table below (*Table 14*).

Superfog Ltd, Superfog			
Formulation	MSS 50M	MSS 50M	Exhaust
Application temperature	350	450	450
Temperature conditions	Lower	Optimum	Optimum

Table 14 Details of treatments conducted for trials to verify ethylene is produced by the thermal fogging application process

The stores were kept entirely sealed prior to venting at approximately twenty-four hours after fog application terminated. After venting the stores were maintained in a 'leaky' state, with some air exchange expected with the ambient surroundings.

The headspace of the stores was sampled for CO_2 and C_2H_4 concentrations (C_2H_4 by Gastec and CO_2 by HiTech landfill gas meter). However for this trial the storage time was

extended to allow sampling for CO_2 to take place over a two-week period (C_2H_4 sample times were all before deliberate ventilation). The pattern of CO_2 levels was monitored to determine if, in normal conditions of storage after CIPC treatment, CO_2 was likely to accumulate to detrimental concentrations. The earlier work indicated that deliberate ventilation would rid the store of any remaining C_2H_4, thereby rendering sampling for the compound useless after this time.

The CO_2 concentrations in the store atmospheres are detailed in the table below (*Table 15*). These are the highest levels measured throughout the entire three-year project. Still they are not sufficient to cause the darkening of fry colour common to tubers that have recently been fogged with CIPC (*Briddon & Jina, 1999*).

Carbon dioxide concentration in store atmospheres (%)			
Sample time (hours post-fog treatment)	Treatment		
	MSS 50M 350	MSS 50M 450	Exhaust 450
0	0.0	0.0	0.0
20	0.6	0.7	0.5
25	0.7	0.8	0.6
96	0.6	0.7	0.7
192	0.7	0.7	0.9
264	0.7	0.5	0.4
360	0.6	0.5	0.4

Table 15 Carbon dioxide concentrations in store atmospheres over a two-week period following fogging

Each treatment caused an increase after fogging, with the same pattern. Slightly enhanced levels over the initial forty-eight hours then a small decline towards the end of the two weeks to approximately 0.5% CO_2 in air. By comparison with the earlier trials when no crop was present in the stores that were fogged, it can be construed that these higher levels are due to respiration of the crop.

The results in the table below (*Table 16*) verify that the application process under normal operating conditions produces C_2H_4. Both CIPC treatments and the exhaust only application generated C_2H_4.

Ethylene concentration in store atmospheres (ppm)		
Sample time (hours post-fog treatment)		
Treatment	**2 h**	**27.5 h**
MSS 50M 350	0.2	0.2
MSS 50 M 450	0.2	0.2
Exhaust 450	1.0	1.0

Table 16 Ethylene concentration in store atmospheres following fogging

The highest concentration was found in the store that received only the exhaust gases (encompassing the hot air from the machine). There was no decline in C_2H_4 concentrations over the twenty-eight hour sampling period. Again C_2H_4 persisted in the store air for a relatively long time for a volatile compound.

Having resolved that C_2H_4 arises from the generation of hog fog the next logical step was to try to qualify the impact this practice has on processing quality.

4.1.6 The effect of thermal fog application on the processing quality of stored potatoes

To determine the effect on processing quality, fry colour assessments were carried out before and after thermal fog treatments. The trials were conducted with previously treated (three CIPC treatments) cultivar Saturna crop in twelve tonne stores at SBEU. The stores were held at 10°C and 95% relative humidity.

All fog applications were conducted using a SCC, Unifog machine, by a qualified applicator (Nick Green, SCC). The flow rate of the MSS 50M formulation through the machine was 1l/min. The dose of CIPC applied and therefore the duration of fogging was calculated based on a full twelve tonne load of potatoes. The run time was thirty seconds for each application. The treatments are outlined in the table below (*Table 17*).

Stored Crop Conservation, Unifog			
Formulation	**MSS 50M**	**Exhaust**	**Untreated**
Application temperature	475	475	n/a

Table 17 Treatments conducted to determine the effect of thermal fog application on processing quality

Each application type was repeated in two separate twelve tonne stores. Fogged crop was transferred to the store holding untreated crop twenty-four hours after application (simulating ventilation). The stores receiving fog treatment contained two individual samples per sampling occasion, while the untreated store contained four pseudo-replicate samples per occasion. Replication of treatments was restricted by store availability. Sample and treatment replication, were both restricted by crop availability and cost.

A sample consisted of a 5kg tray with approximately thirty tubers. From these a representative subsample was selected, made into crisps as per British Potato Council (BPC) standard operating procedure. The fry colour was assessed instrumentally by the Hunter Lab system and subjectively using a light cabinet and colour reference cards.

The headspace gas of the treated stores was monitored for CO_2 and C_2H_4 preceding ventilation (C_2H_4 Gastec and CO_2 by HiTech landfill gas meter). The mean atmospheric gas concentrations are presented in the following tables (*Tables 18 and 19*).

Carbon dioxide concentration in store atmospheres (%)		
Sample time (hours post-fog treatment)		
Treatment	8 h	24 h
Untreated	0.0	0.0
Exhaust	0.3	0.5
MSS 50M	0.3	0.5

Table 18 Carbon dioxide concentration in store atmospheres following treatment

The accumulation of CO_2 is greater in stores that received an application from the fogging machine. Both the CIPC formulation and exhaust treatments generated an equal amount of CO_2 in store air. However these levels are relatively low and are not consistent with a darkening of fry colour.

The C_2H_4 concentration was not determined in the untreated stores.

Ethylene concentration in store atmospheres (ppm)		
Sample time (hours post-fog treatment)		
Treatment	8 h	24 h
Exhaust	5	5
MSS 50M	5	5

Table 19 Ethylene concentration in store atmospheres following fogging

Both application types caused an accumulation of C_2H_4 in the store atmospheres to the same extent. C_2H_4 was detectable for the full twenty-four hours preceding ventilation. It is clear that the current standard fogging procedure with or without a CIPC formulation will introduce C_2H_4 into potato stores in the approximate range 1-10ppm. C_2H_4 generation is symptomatic of combusting hydrocarbon fuel, particularly petrol.

The trial was conducted quite late on in the storage season hence the relatively low baseline fry colour (trial started May 2001). The graph (*Figure25*) displays the Hunter L-value. This is a measure of the brightness of the crisps. The higher the L-number the better the quality of the fried sample. The value plotted is the mean of each treatment. The error bars show plus and minus one standard deviation.

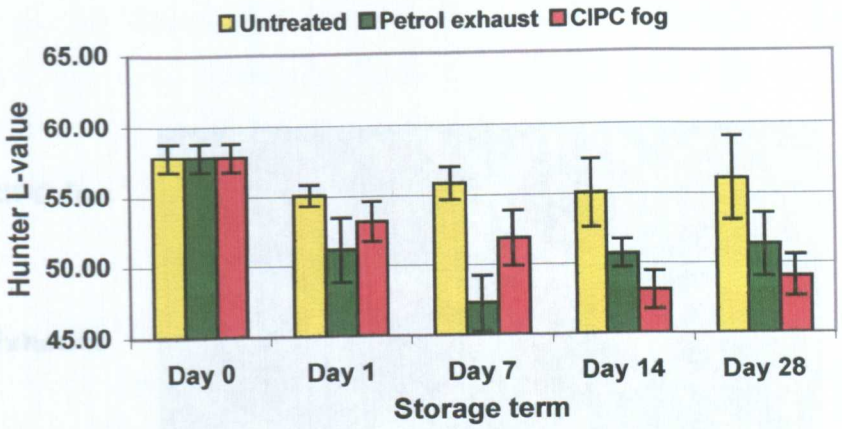


Figure 25 The effect of thermal fogging on fry colour during storage (n=4)

Throughout the twenty-eight days of storage untreated samples consistently had the lightest fry colour. Thermal fog application caused a darkening of fry colour. Samples of tubers after exhaust only and CIPC fog treatments had reduced processing quality and less market acceptability for the duration of the trial. The greatest impact on fry colour was on day 7 or 14 of storage depending on treatment. After the lowest L-value was reached the fry colour appeared to improve to some extent. Potentially the crop was beginning to recover from the stress of the thermal fogging.

Treatment	Day 1	Day 7	Day 14	Day 28
Untreated	a	a	a	a
Exhaust	ab	b	b	b
CIPC	b	c	b	b

Table 20 Statistical analysis of L-values. Different letters denote significant differences

The effect of thermal fog treatment is evident. The untreated crop was of a superior quality when the trial ended. The length of the storage period is important as the legal time interval between CIPC treatment and crop going to market is 21-28 days, depending on formulation.

Below is a photograph of some of the crisp samples from this trial. It illustrates the clear effect of the thermal fogging process on fry colour.

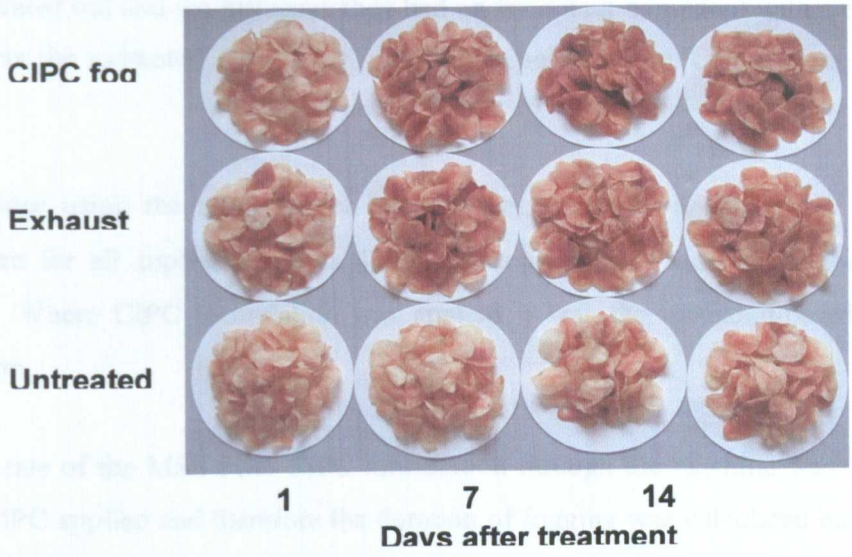


Figure 26 The effect of the thermal fogging process on the aesthetic quality of crisps

The conditions of storage were different for the untreated crop. The only CO_2 present was from respiration of the potatoes. The store was not checked for C_2H_4 content, however previous trials have shown that any C_2H_4 present would be naturally occurring from the potatoes in much lower levels (approximately by a factor of 1000). At these concentrations C_2H_4 is not expected to cause darkening of fry colour. In the critical twenty-four hour period between application and ventilation the balance of gases in the store atmosphere was different. The untreated potatoes were not exposed to the stressful situation created by thermal fog treatment. These results denote the importance of the environmental conditions of storage, particularly in the crucial stage between application and ventilation.

From this point on greater emphasis was placed on the presence of C_2H_4 in store atmospheres than CO_2. All the measurements of CO_2 taken have been less then 1.0% of the store air. At concentrations of less than 1.5% there will be no detrimental impact on fry colour. The subsequent trial investigated processing quality focusing on C_2H_4 concentration in stores and the related fry colour of processed samples.

The second trial assessing the effect of thermal fogging on the processing quality of potatoes was conducted at SBEU. The aim of the trial was to confirm that the reason for the typical dark fry colours after treatment is the application process. The design is outlined in the table below (*Table 21*). All the individual stages of the application process were separated out and the influence they had on fry colour compared with a control. The control was the untreated crop, which was not exposed to any output from the fogging machines.

One operator using the same fogger machine conducted all applications. The burner temperature for all applications was 475°C, the optimum as selected by the machine's operator. Where CIPC formulation was applied it was the methanol-based MSS 50M formulation.

The flow rate of the MSS 50M CIPC formulation through the machine was 1l/min. The dose of CIPC applied and therefore the duration of fogging was calculated based on a full twelve tonne load of potatoes. The run time for each application was thirty seconds.

Stored Crop Conservation, Unifog	
Treatment 1	Untreated
Treatment 2	Exhaust
Treatment 3	Exhaust + methanol
Treatment 4	CIPC (8 hour ventilation)
Treatment 5	CIPC (24 hour ventilation)

Table 21 Treatments conducted to assess which stage(s) of the application process has the detrimental impact on processing quality.

Cultivar Saturna was used. The crop temperature was maintained at 10°C and the store held at 95% relative humidity prior to treatment. Treated crop was transferred to the store holding untreated crop at the pre-determined time after application (simulating ventilation). In most cases this was twenty-four hours after treatment. In the case of treatment 4, ventilation was performed eight hours after application terminated. For treatment 3, the methanol referred to is the volume equivalent of the methanol that would be applied as part of the CIPC formulation (52%w/v). The actual solvent applied was technical grade methanol bought in specifically for this trial.

Due to restrictions in store availability and cost there was no replication of treatments in this trial. Each treatment had two individual samples per sampling occasion.

The resultant fry colour was assessed instrumentally by the Hunter Lab system and subjectively using a light cabinet and colour reference cards. A sample consisted of a 5kg tray with approximately thirty tubers. From these a representative subsample was selected, made into crisps as per British Potato Council (BPC) standard operating procedure.

The headspace gas of the treated stores was monitored for CO_2 and C_2H_4 preceding ventilation (C_2H_4 by Gastec and CO_2 by HiTech landfill gas meter).

CO_2 was introduced to all stores that received treatment by the fogging machine, in comparable concentrations whatever the stage of the process. All levels prior to ventilation were 0.3-0.4% (excluding untreated, 0.0%). Similarly, in all stores that received a treatment involving the fogger machine ethylene was detected (concentration range 1-5ppm).

The graph below (*Fig 27*) displays the fry colour results in terms of L-value (the brightness of the sample). The higher the L-number the better the quality of the crisp sample. The value plotted is the mean of each treatment. The error bars show plus and minus one standard deviation. In particular samples the standard deviation is rather high. This is because of the inherent variability of the natural product. A greater number of samples would have been much preferred. However the overall trends are in line with all the previous results and therefore considered reasonably representative.

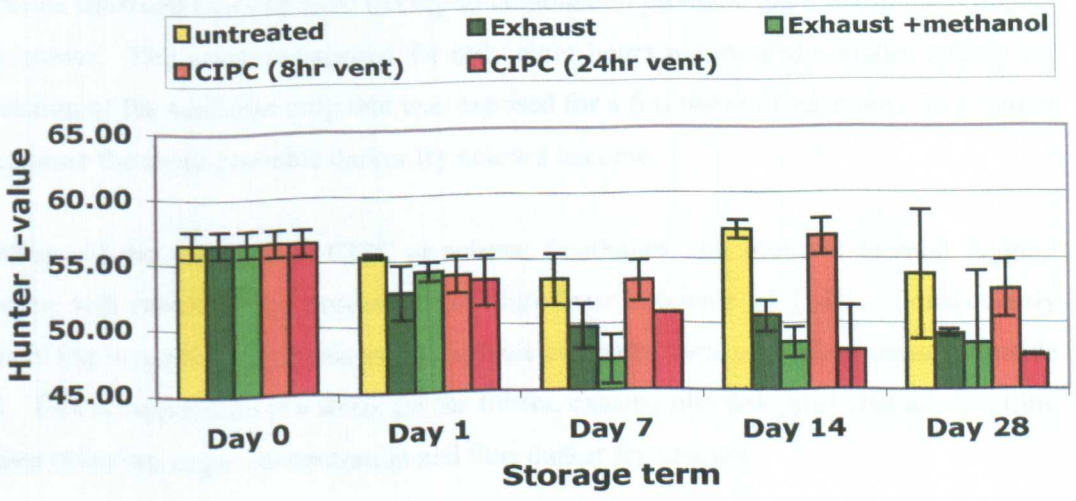


Figure 27 The effect of the fogging process on fry colour during storage (n=2)

Treatment	Day 1	Day 7	Day 14	Day 28
Untreated	a	a	a	a
Exhaust	a	a	ab	a
Exhaust + Methanol	a	a	b	a
CIPC (8hr)	a	a	a	a
CIPC (24hr)	a	a	b	a

Table 22 Statistical analysis of L-values. Different letters denote significant differences

Each stage of the fogging process was detrimental to the processing quality of the potatoes. Separating the stages demonstrated quite effectively that the basic problem with thermal fogging is combusting hydrocarbon fuel and exhausting it into stores. This feature was common to all applications (excluding the untreated crop). Each application had a negative effect on fry colour.

Exhaust only treatment was initially more damaging to crop, suggesting exhaust disturbs crop more rapidly than fog (with solvent and/or CIPC). Assuming the addition of solvent reduces the temperature of the flow into store, the exhaust only would have been the warmest treatment applied. It is widely accepted that higher fog temperatures are more harmful to potatoes in storage.

The results illustrate that exposure of crop to combustion products has a detrimental impact on fry colour. The samples exposed for only eight hours maintained a higher quality for the duration of the trial than crop that was exposed for a full twenty-four hours. The longer the exposure the more probable darker fry colours become.

Regardless of the presence of CIPC or solvent (methanol) the standard thermal fogging procedure will result in the production of significant amounts of C_2H_4. Consequently wherever fog is applied using this system sizable concentrations of C_2H_4 accumulate inside stores. This accumulation is a stress for the tubers, causing physiological changes resulting in higher reducing sugar concentration and thus darker fry colours.

4.1.7 Features of the thermal fog application process that can potentially alter the quality of stored potatoes

The C_2H_4 produced by the process of thermal fogging is a consequence of incomplete combustion of petrol. The amount of C_2H_4 generated is dependant on the following factors:

- Efficiency of combustion
- Petrol consumption
- Duration of application

The efficiency of combustion will be less than 100% as with all mechanical equipment. This is not expected to vary extensively between individual fogger machines. The three representative commercial foggers investigated were all based on the same principles of fog formation. Each machine was optimised to the operator's satisfaction, in which case efficiency of combustion would be a priority and as close to 100% as possible.

Petrol consumption is a function of not only the efficiency of combustion but also the application temperature. This will vary between machines as each application company has its own optimum temperatures for use of specific CIPC formulations. The higher the burner temperature the more energy required to achieve this. A greater energy output consumes more fuel. The more petrol consumed and the lower the efficiency of the burner the more C_2H_4 will be produced.

The duration of application is pre-determined by the dose of CIPC being applied. The flow rate of CIPC formulation through the delivery nozzle into the fog head is common to most machines (assuming a standard size delivery nozzle is used). It is formulation specific, based on the viscosity of the carrier, e.g. MSS 50M (methanol-based) formulation has a delivery flow rate of 1l/min, whereas Warefog (methylpyrolidone-based) formulation has to be delivered at 0.8l/min because it is a thicker solvent.

The flow rate of the fog, the total volume entering the store and the temperature are all key features that are likely to influence the effect of the fog on the potatoes.

Flow rate is a function of the speed of the fog as it leaves the pipe (metres/sec) and the diameter of the exit port of the pipe. The flow rate of the hot air stream and the combustion gases was calculated using this information. The anemometer used to measure the speed was limited to use in temperatures below 80°C. Therefore the speed could not be measured at normal application temperatures. The range calculated includes the flow-rate as a result of air pulled in by the blower and the petrol combusted to run the engine (~50°C). The flow-rate of the CIPC fog would be faster as more combustion gases would be present from burning a greater volume of fuel to achieve application temperature.

The flow-rate of air from the blower measured for this work was found to be in the range 700-800m^3/hour. This range is based on the most representative and reproducible speed determined in trials that were conducted with only the petrol required to run the engine (temperature range of 45 to 55°C). When all the combustion gases and volatilised CIPC formulation are included the value is expected to increase to nearer 1200m^3/hour *(Dr H. Duncan, personal communication)* under normal application conditions. 1200m^3 includes the volume of combustion gases created by burning the petrol required to facilitate fog production at an approximate burner temperature of 400 to 500°C.

Thermal fog application into a 2000 tonne store will normally take one hour including set up time (to reach the necessary burner temperature). This of course depends on the delivery flow rate of the CIPC formulation applied. One hour is characteristic for application of MSS 50M (methanol solvent). The delivery flow rate of which is 1l/min. At a dose rate of 28.5ml/tonne for 2000 tonnes 57 litres would be applied. This rate is for the second and subsequent application throughout a storage season (the dose rate would be greater for the first application of the season). Thus the estimated volume of gas entering a 2000 tonne box store per fog application is 1200m^3.

Previously measured dimensions of a 2000 tonne box store show that the air inside a full store is approximately 76% of the entire store volume. The parameters below were used to ascertain what the volume of fog relative to the volume of air inside a full store is. The total volume of air inside a store that will be involved in displacement by fog and diluting fog components includes free air and the air within each box.

4.1.7.1 Parameters of a full 2000 tonne box store:

Total store volume: $7580m^3$

1 tonne box volume: $2.05m^3$

1 tonne of tubers occupies: $1.5m^3$ (of that $0.9m^3$ are tubers and $0.6m^3$ is air space between tubers)

Air space within a 1 tonne box: $2.05-0.9=1.15m^3$

Total space occupied by boxes: $2000\times2.05=4100m^3$

% of store containing free air: $7580-4100=3480m^3$, $3480\div7580\times100=45.9\%$

Free air within volume occupied by 2000 boxes: $1.15\times2000=2300m^3$

Total air inside the store: $3480+2300=5780m^3$, $5780\div7580\times100=76.3\%$

The above data illustrates the proportion of air displaced by fog during application is quite large.

$1200\div5780\times100=\underline{20.8\%}$

A 20% proportion of the store air being replaced with a warmer mixture of gases (including CIPC particles) in a one-hour interval could potentially disturb the quiescent state of the potatoes. Regardless of the composition of the fog the introduction of a temperature difference, albeit short-lived, is unfavourable (*Duncan et al, 2001*).

Formerly the temperature of the fog as it leaves the application pipe was believed to be in the region of 100°C for burner temperatures of 400 to 500°C. The measurements of temperature (exhaust only, no CIPC formulation) taken under these conditions found that the gas at the outlet was in excess of 200°C. The addition of CIPC formulation will reduce the temperature of the flow because of the solvent properties. The degree of cooling has not been measured because of temperature limits on the equipment used and safety restrictions. Nonetheless it is doubtful that the formulation will bring the outlet temperature down to less than 100°C.

In conclusion, investigation of the thermal fogging application process raises many questions over its suitability for application of a sprout suppressant to stored potatoes. There are many aspects, which are unfavourable for preserving crop in a dormant state. The main concerns are a) C_2H_4 production and the resultant exposure of tubers to this b)

gases at elevated temperatures entering stores at rapid rates and c) The frequency of reapplication creating potentially stressful situations at regular intervals.

The positive attributes of the current application procedure are a) the speed and ease of application and b) knowledge of the expected CIPC particle size produced and some level of control over this. Also for a number of years the distribution of the CIPC inside the store has been the subject of considerable research, particularly now as the impending Maximum Residue Level increasingly gains significance and attention within the food industry.

4.1.8 The Combustion process

Fossil fuels are formed over millions of years through the decay and compaction of rotting organic material, both on the land and on the sea floor. The conditions of temperature and pressure over this extensive time period have formed three types of fuel Coal (from vegetation), oil and gas (both from marine organisms). Burning fossil fuels releases a number of pollutants, including sulphur dioxide, nitrogen oxides, carbon monoxide, particulate matter and volatile organic compounds, including hydrocarbons. Petrol is a hydrocarbon fuel refined industrially by fractional distillation of crude oil. It will burn in air or oxygen when an ignition source is present. As with all hydrocarbons combustion of petrol is a highly exothermic reaction. Lead replacement petrol is burned in air to heat the cold air stream and enable thermal fog to be created.

Ideally pure hydrocarbons will be oxidized completely on burning. If this were the case the major products of combustion would be water vapour (H_2O) and carbon dioxide (CO_2). To achieve this the correct fuel/air ratio would have to be found and adjusted as necessary for precise oxygen fluctuations. A plentiful supply of oxygen is a prerequisite for complete combustion. The general rule for determining oxygen demand is the greater the number of carbon atoms the more oxygen will be required and hence the more energy released. Insufficient oxygen supply leads to the production of carbon monoxide (poisonous), or in an extreme case carbon (soot build up in the system can lead to explosions). Inadequate ventilation is the main reason for incomplete combustion. Other times when the products of incomplete combustion dominate are when engines are idle, at slow speeds or warming up.

In contrast to the above at high temperatures more pollutants can be formed. For example atmospheric nitrogen can be oxidised through nitrogen monoxide (NO) to nitrogen dioxide (NO_2) at high temperatures. On burning, impurities in the fuel are also oxidised to potentially harmful products.

Petrol is not a pure alkane. The fractional distillation process separates hydrocarbons based on boiling point (molecular weight). The lighter the molecule the more pure the fraction that can be collected. As petrol is a moderately heavy fuel it is an inherently mixed fraction, consisting of a range of hydrocarbons all within a similar molecular weight range (5-11 carbon atoms). The set back with burning petrol is the range of compounds produced (benzene, polyaromatic hydrocarbons, unburned hydrocarbons, nitrogen oxides etc). This is the fundamental reason why automobiles are fitted with catalytic converters.

The chemical formula of typical automobile petrol is C_7H_{16}. However petrol is known to contain a wide range of different hydrocarbons and therefore the mean formula of this fraction of the distillation process can be represented as $C_{7.3}H_{15.8}$. The reaction below is for complete combustion of petrol.

$$C_{7.3}H_{15.8} + 11.25O_2 \rightarrow 7.3CO_2 + 7.9H_2O$$

Balancing the equation shows that 11.25moles of oxygen are required for complete combustion of each mole of petrol. Assuming combustion is complete, each mole of petrol will yield approximately 7.3 moles of carbon dioxide and 7.9 moles of water vapour.

To convert the molar values of combustion products into volume of gases certain assumptions are necessary, as below:

The mean fractional formula of petrol ($C_{7.3}H_{15.8}$) is representative. The exact content of crude oil and therefore petrol is changeable depending on its starting material and formation. The above chemical formula is generally acceptable as an indicator of what petrol is and how it will perform under various conditions.

Combustion is complete. Hence, the moles of combustion products per mole of petrol are correct. In reality combustion will not be complete and volatiles of a contaminant nature will be produced, but the extent of this will dependent heavily on the engine in use. Because of the variable nature of this factor it is excluded from the calculations of combustion gases. The values calculated are the best-case scenario.

The conditions of temperature and pressure throughout an application are constant. Experience with fogger machines has shown there are consistently small fluctuations in both burner temperature and combustion chamber fuel pressure. The control panel is monitored and adjustments made to stabilise the running conditions during this time. Therefore on balance the desired burner temperature and resultant combustion chamber fuel pressure are used with confidence in the calculations that follow. 500°C is used in the example overleaf as the fuel consumption was determined experimentally at 500°C and 400°C.

The ideal gas law allows the volume of a gas to be calculated at specified conditions of temperature and pressure.

$$V_m = R \left(T/P \right)$$

Equation 1 Ideal gas law

V_m=Molar volume (l/mole)

R=Universal gas constant (0.0831447 bar/l)

T=Temperature (°Kelvin)

P=Pressure (bar)

The conditions of a typical CIPC application to a 2000 tonne box store are:

T=500°C≡773°K

P=2.7bar

V_m= (773÷2.7)

V_m=23.80l/mole

Under these conditions of temperature and pressure 23.80l/mole of carbon dioxide and 23.80l/mole of water vapour will be produced for every mole of petrol combusted.

Taking account of the yield of these components (7.3 and 7.9 moles for CO_2 and H_2O respectively) the total volume of combustion gases will be 361.78l per mole of petrol.

(7.3×23.80=173.74l) + (7.9×23.80=188.02l) = 361.78l/mole of petrol

The number of moles of petrol consumed during application is determined from the volume of petrol used and the specific gravity of petrol at ambient temperature (standard conditions of temperature and pressure, 25°C and 1atmosphere ≈1 bar). The specific gravity of lead replacement petrol is ~0.73g/ml and mean measurement of petrol consumption for one hour of fogging at 500°C is 12.840litres. The value of consumption

is from the table of the measured range of fuel consumption under specific conditions (*Table 23).*

The molecular weight of petrol is 103.4 atomic mass units; therefore one mole of petrol has a mass of 103.4g.

103.4g÷0.728g/ml=142.033moles/ml

12840ml petrol÷142.033moles/ml=90.402moles of petrol

90.402moles of petrol×361.78l/mole=32705.46l of combustion gases

From this the total volume of combustion gases produced during a typical one hour fogging operation is 32705.46l. Of this 15706.44l is carbon dioxide.

173.74l CO_2/mole of petrol×90.402moles=15706.44l carbon dioxide

The volume of air in a typical full 2000 tonne box store is estimated at 5780m^3. This equates to 5780,000l of air. Based on these assumptions the fraction of store air occupied with carbon dioxide after fog application is 0.27%.

(15706.44÷5780000)×100=0.27%

This concentration would not be reached in reality (not including CO_2 from respiration) as the store has to be opened to some extent (leaky) to prevent pressurising it by forcing a further ~20% gas into the fixed volume store. Therefore some fog including CO_2 is lost to the surrounding environment.

0.27% CO_2 illustrates the maximum concentration arising from thermal fog application under these conditions. Incomplete combustion (normal) would generate a lower concentration. A few of the CO_2 values measured throughout this project have been greater then 0.27%, indicating that a fraction of it was from respiration. The background CO_2 levels recorded were on most occasions 0.0%. In this circumstance it is reasonable to assume that CO_2 recorded between 4-24 hours after thermal fog application in excess of 0.27% is partly from respiration of crop in response to fog treatment and sealed storage until ventilation.

4.1.9 Fuel consumption for lead replacement petrol

The fuel consumption was measured at different times of the year to account for the influence of ambient conditions on the fuel demand. SCC Unifog machines were used. Measurements were taken over a fifteen-minute period that started when application temperature had been reached. Firstly the fuel used to run the engine (no load) was measured, then to run the fogger at the desired temperature. From this only the fuel needed to create combustion gases was calculated. The burner temperatures tested are representative of the range in use for a methanol-based CIPC formulation.

The values stated in *Table 23* are for the burner temperature only; the value for engine usage is shown and has already been subtracted.

Date	Unifog	Burner temperature (degrees C) engine only	400	500
Jul-00	B	880	2420	3120
Apr-01	A	700	2475	3100
Apr-01	B	800	2700	3575
Jun-02	A	830	1550	2170
		mean:	2286	2991
		St Dev:	505.6	589.8

Table 23 Volume of petrol consumed in a fifteen-minute period (ml)

The difference in efficiency of machines is evident from results for measurements taken in April, when ambient conditions were the same while testing machine A and machine B. The difference in fuel consumption between the machines is a consequence of the extent of combustion. Both machines are fitted with the same engine, however Machine B was not performing as well. The time since the last service or the workload of the machines may have been different. The fuel consumption was less when measured in June and July when the ambient temperature was higher. The smaller the difference between ambient and application temperature e.g. 20°C to 500°C, compared with 11°C to 500°C, the less energy needed to achieve this.

The mean value for petrol consumption at application temperatures of 400 and 500°C is 2286.25l and 2991.25l respectively for a fifteen minute period. For an application that would take one hour to complete the total lead replacement petrol consumption is likely to approximate to 119651 at 500°C.

4.1.10 Alternative fuels

Alternatives to petrol were considered as starting materials for generating thermal fogs because they could potentially reduce the amount of contaminants entering a store, thereby benefiting fry colours.

Therefore fuel consumption was measured for both Methanol and Liquefied Petroleum Gas (LPG) using the same type of commercial Unifog machines (SCC). Modifications had to be made to the machinery for both fuels. The methanol system was ready for use quite rapidly as the modifications to the combustion chamber and the fuel delivery nozzle were straightforward and effective from the start.

The modifications required to burn a pressurised gas were vastly more complicated. The first attempt was sufficient to provide a short run time for application of CIPC to experimental stores (see chapter 7). Latterly it was revealed that the burner in this system was at most 30% efficient, therefore not indicative of what a change to a LPG fuel source could mean in terms of fry colour. The fuel consumption values shown are those measured with the first LPG test machine, when efficiency of combustion was very low.

The supply of LPG was from a domestic (BBQ, propane only) pressurised LPG cylinder delivered at a pressure of ~2bar to the fogger combustion chamber. This supply was sufficient for a burner temperature of 400°C, however would not provide an adequate rate of supply to reach 500°C. An industrial forklift truck cylinder (held at a much higher pressure) was fitted onto the regulator and attached to the machine. This permitted the higher temperature to be reached, however because the efficiency of combustion was poor a considerable volume of unburned fuel was passing through the flame trap. As a result there was flashing of the fuel as it exploded at the end of the fog head. The value for 500°C cannot be considered characteristic for LPG applications under any circumstance and is therefore not included. The practical testing of the equipment demonstrated the importance of having the correct working parameters for safe combustion of fuel.

The fuel consumption was measured for a fifteen-minute period that started when application temperature was reached. As with petrol the fuel used to run the engine was subtracted from the fuel required to maintain the desired burner temperature. Hence only the fuel entering the store as combustion products was calculated.

Date	Unifog	Burner temperature (degrees C) 400	500
Jun-02	A	5270	6000

Table 24 The volume of methanol consumed in a fifteen-minute period (ml)

LPG use was measured by weighing the cylinder at the start of the fifteen minutes then again after the run. The pressure inside the cylinder was constant providing the cylinder was not allowed to empty completely.

Date	Unifog	Burner temperature (degrees C) 400
Jun-02	F	2615.5

Table 25 Weight of LPG (propane) consumed in a fifteen-minute period (g)

Knowing the ambient temperature and the operating pressure in the domestic cylinders the weight of LPG used can be converted into a volume of gas (m^3). However quite often cylinders are sold by the mass of propane inside them. The pressure information provided is limited and only details whether vapour or liquid LPG is being drawn off for use. High-energy outputs require liquid use.

4.1.11 Predicted levels of ethylene introduced to store atmospheres by thermal fog application

Using the results obtained on petrol consumption, application time and fog volume entering stores it is possible to postulate the concentration of ethylene in store atmospheres resulting from fogging.

It is previously known that a small proportion of all hydrocarbon combustion gases will be ethylene. A suggested figure of 0.12% (*Sinclair, 1998*) is used for the purposes of calculations to illustrate potential levels in thermal fogs.

The volume of combustion products produced in a typical one hour fogging operation for a full 2000 tonne box store at a burner temperature of 500°C is approximately 32705.46l. 0.12% of this is 39.25l.

The total volume fogged into the store in an hour including CIPC particles from the formulation is understood to be approximately 1200m^3(equalling 1200000l).

39.25l÷1200000l=0.00003270837≡32.71ppm

32.71ppm in fog will be diluted by the volume of free air inside the store (approximately 20.8%) yielding a store atmosphere concentration of 6.80ppm.

(32.71ppm÷100)×20.8%=6.80ppm

Upon calculating the estimated concentration of ethylene in the store air for the range of application temperatures and fuel consumption rates the values obtained are all within the range 0-10.0ppm.

In the experimental work conducted ethylene has been detected in appreciable amounts in store atmospheres following fogging (in the region of 0.2-10.0ppm).

10ppm is generally lower than the concentrations quoted in literature of 50-60ppm (*Wang & Pritchard, 1997*). However levels above 0.2ppm are sufficient to induce a physiological response in tubers (*Abeles, 1973*).

4.1.12 The energy created by a thermal fog application

The energy produced by the amount of petrol combusted under a given set of conditions can be calculated using thermodynamic principles. This energy is required to reach and maintain the target burner temperature (plus any losses).

The assumptions in this model are:

That each unit of fuel is combusted at 100% efficiency and will release its full energy potential. The exact efficiency of fuel combustion in the fogger system is unknown (but will be less than 100%).

When the system is optimised for alternative fuel use efficiency it is not expected to vary widely between fuel sources.

Standard conditions of temperature and pressure are adopted when using the physical and chemical properties of the fuels in calculations where possible. These are 25°C and 1atm≈1bar. These are assumed to allow suitable comparisons to be made.

The energy released by the petrol consumed for a fifteen-minute run at both 400 and 500°C will be used as a gauge for the amount of energy required for a typical thermal fog application of CIPC.

The mean volume of petrol consumed for a 15 minutes run at 400°C is 2.286l and the density of petrol=750g/l (at standard conditions).

2.286l×750g/l=1714.68g≡1.7147kg of petrol used in the run

The gross calorific value of petrol=45-47MJ/kg, on average 46MJ/kg.

1.7147kg×46MJ/kg=78.8762MJ

Burner temperature	Energy released in 15 minutes (MJ)
400°C	78.8762
500°C	103.1981

Table 26 The energy produced by the combustion of petrol (based on an average value of petrol consumption at each burner temperature)

This is how the volume of fuel required to release this known amount of energy is resolved for different fuel sources.

The gross calorific volume of methanol is 23MJ/kg

At 400°C: 78.8762MJ÷23MJ/kg=3.4294kg=3429.4g of methanol required

the density of methanol is 791g/l at standard conditions

3429.4g÷791g/l=4.335l

For propane (LPG) the calculation has to be conducted on a molecular basis because it is a gas.

The gross calorific value of propane is 94MJ/m^3

78.8762MJ÷94MJ/m^3=0.83911m^3

the molar volume of propane at standard conditions is 0.025m^3

0.83911m^3÷0.025m^3/mole=33.564moles required

The density of propane at the cylinder conditions is 518.84g/l and the molecular weight of propane is 44g.

The cylinder contains 19kg in 36.62l at 25°C (*N. Green, personal communication*).

518.84g/l÷44g=11.79moles of propane in every litre

33.564moles÷11.79moles/l=2.8468l

0.8468l≡0.0028468m^3

The cylinder used for the fuel consumption trial at 500°C was larger (suitable for use with a forklift truck). The pressure in this cylinder was greater and therefore the density of propane would have been more than in the smaller cylinder, hence more moles of propane were present per litre. So overall less volume from this source would be required to provide an equal amount of energy from the smaller cylinder. The rate of fuel delivery is increased in the large cylinder because the regulator used is different. This increased rate of fuel injection was necessary for the burner temperature to reach 500°C.

Burner temperature	Petrol (ml) *	Methanol (ml)	Propane (ml)
400°C	2532	4335	28468
500°C	3265	5672	

*Measured value based on overall mean for fuel consumption assessed in different seasons of year

NB. Gas (propane) volumes are normally expressed as m^3. To get m^3 divide Litres by 1000.

Table 27 The volume of fuel required to run a thermal fogging machine at the specified burner temperature for a fifteen-minute period.

Theoretically the volume of methanol required to generate a fog is much greater than that of petrol.

The table below (*Table 28)* compares the actual measured methanol fuel consumption with the theoretical calculated value. The values for 500°C are reasonably close. However the difference in volumes at 400°C is considerable for a fifteen-minute run time. The measured value being greater than the theoretical illustrates the efficiency of combustion was less than 100%. The larger difference at 400°C indicates the efficiency was lower under these conditions. This temperature is not as high as it was at 500°C when the values were within reasonable agreement.

Burner temperature (Degrees Celsius)	Volume of methanol (ml)		Difference in values (measured-theoretical)
	Theoretical	Measured	
400	4335	5270	**935**
500	5672	6000	**328**

Table 28 Comparison of methanol volumes measured and theoretical for fog generation.

4.1.13 Conclusions

These findings demonstrate that standard generation of a thermal fog used for CIPC application introduced ethylene to the potato store. The actual measured values, of between 0.2 – 10.0ppm, compared very well to the calculated theoretical value of 6.8ppm. Depending on ventilation and air exchange it could remain present in headspace for at least twenty-four hours. This was shown to be deleterious to the fry colour of crisp samples collected for a number of weeks following application.

This is in agreement with *Blenkinsop et al, 2002,* who showed that CIPC does not affect fry colour but the application of fog can.

The information on specific aspects of thermal fogging machines opens up avenues for potentially reducing or removing this detrimental side effect. This information includes:

- The expected volume of store air being displaced during a typical application at approximately 20%

- The maximum concentration of CO_2 generated during a typical application was calculated as 0.27%

- To achieve and maintain the same application temperature as a normal petrol fuelled application approximately 1.5 times the volume would be required for methanol and very approximately 10 times the volume would be required for propane (it should be borne in mind that propane is supplied as a pressurised gas source).

The following chapter focuses more closely on the influence that the compound ethylene has on potato tubers relevant to processing quality.

A major concern surrounding pesticide application is the frequency with which it is applied by unqualified and in some cases even untrained persons. This is a particular issue with CIPC use. Many store managers either apply CIPC themselves with Swingfog machines or hire a local Swingfog applicator to conduct treatments. This is not recommended as it can lead to frequent poorly monitored applications. It is a less expensive option but often the applicator does not have developed knowledge and experience of potatoes. Although as highlighted, there is clearly room for improvement with current thermal fogging machines

as they are recommended for the best practice of CIPC treatment during long-term storage. A license has to be obtained for commercial operation of this type of equipment.

A classification scheme of pesticide application equipment and its potential contamination hazards, was proposed by the British Crop Protection Council (*Parkin et al, 1994*). This was designed to inform the user and aid decisions toward reducing the potential hazards during use. Similarly a European community initiative on inspection and certification of pesticide application equipment was implemented (*Ozkan, 1999*). Ozkan suggested that improvement in applicator training be made and the possibility of license renewal times be considered. The increasing legislative control of pesticide use means that many European countries are introducing procedures to control and improve operator skills and the quality of equipment used in pesticide applications (*Friedrich, 2000*).

5 Chapter Five

5.1 IDENTIFYING WHEN PROBLEMS OCCUR AND VERIFYING THAT ETHYLENE HAS AN EFFECT ON PROCESSING QUALITY

5.1.1 Background Information on Ethylene

Previous literature has shown that ethylene is central to the deterioration in processing quality of potatoes following fogging (*Wang & Pritchard, 1997*). Data from this project has drawn the same conclusion. However the actual effects of ethylene on potato tubers had to be investigated.

Ethylene is a colourless flammable gas at room temperature with a sweet odour. It has two carbon atoms and a double bond. Its molecular weight is 28.05atomic mass units. It has explosive limits of 2.75-28.60% in air (*Abeles, 1973*)

Ethylene is one of the simplest organic molecules with biological activity. It is a plant hormone that regulates many aspects of plant growth, development and senescence (*Abeles et al., 1992*). It is produced naturally in all higher plants in essentially all tissues. The production of ethylene varies with type of tissue and stage of development. It is important to physiological regulation of plants and, in particular, senescence and post-harvest physiology of vegetables.

Potato tubers are very sensitive to ethylene and their response to changing levels is dependant on exposure time, concentration, cultivar sensitivity, atmospheric composition and temperature (*Loughheed, 1987*). An increased ethylene level stimulates respiration, accelerates senescence and alters sprouting (*Saltveit, 1999*)

Ethylene is produced in higher concentration in the peripheral layers of the tuber, where cell division (mitosis) occurs. The rate of production increases with the advance of growth of sprouts (*Okazawa, 1973, Suttle, 1996*). Any physiological or mechanical stress will cause an increase in production of this natural plant hormone in response (*M'Glasson, 1969, Moore, 1979, King & O'Donoghue, 1995, Bleeker & Kende, 2000*). Both ethylene synthesis and sensitivity are enhanced during certain stages of development and by a number of biotic and abiotic stresses

Ethylene production in potato tubers is normally negligible at less than $5x10^{-4}$µl/kg/hr at 7°C, but can increase to $3x10^{-1}$µl/kg/hr at the same temperature if the tubers are exposed to stressful conditions (*Breech et al, 1973*). An increase by a factor of 1000 can be expected (*Burton, 1989*). *Burton and Meigh, 1971* stated that the production rate of ethylene in potatoes was in the order of 1ng/kg/hr under normal storage conditions and as such did not form an important component of the total volatiles exuded. They also showed that if volatiles were allowed to accumulate (no ventilation, exchange with ambient air or forced circulation) for a number of weeks, after ~4 weeks, ethylene would be in the approximate concentration of 0.6µg/l (~0.5ppm) then effects upon growth would be expected. However for sprouting to be suppressed by ethylene the concentration would have to be considerably higher in the order of 10-100ppm constantly in air.

External ethylene is considered a stress to the potato plant. The threshold observed for phytotoxic effects of ethylene under laboratory conditions is $12ug/m^3$ (*Tonneijck et al., 2000*). Harvested vegetables may be unintentionally exposed to biologically active levels of ethylene and both endogenous and exogenous sources of ethylene contribute to its biological activity (*Saltveit, 1999*). Therefore exposure to an external source can induce increased biosynthesis (autocatalytic reaction).

Some important interactions between the plant and its environment that outline how ethylene affects plants are illustrated below (*Fig 28*) (*Saltveit, 1999*).

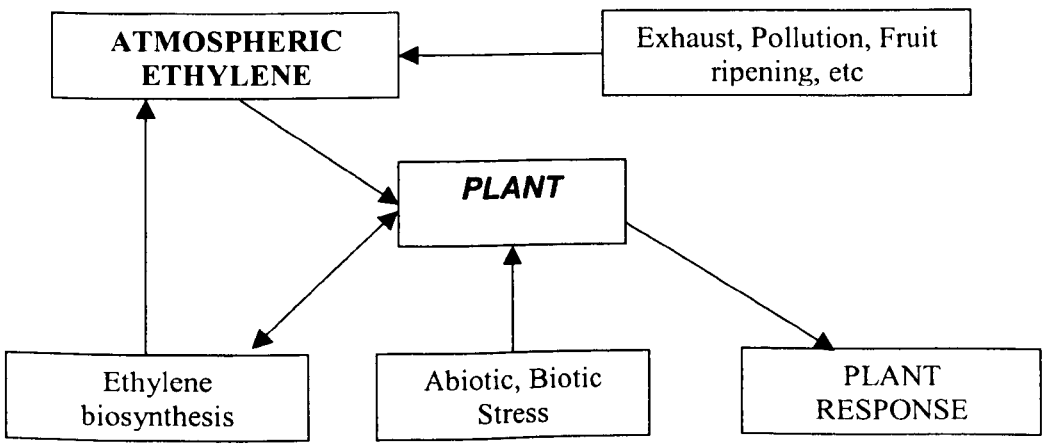


Figure 28 Interactions between the plant and its environment

Plant responses to ethylene are extensive and include accelerated growth, retarded growth, increased respiration, etc.

Ethylene is a small gaseous compound (*Fig 29*) that will move freely in air and can absorb easily onto available sites. The double bond (olefin) makes this a considerably active compound.

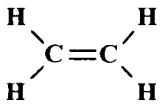


Figure 29 The structure of ethylene

It is effective at very low levels (<0.5ppm), however the concentrations produced from stored tubers are too low for active sprout inhibition as previously discussed (*Rylski et al, 1974, Burton and Meigh, 1971*).

5.1.1.1 Natural biosynthesis of Ethylene

Ethylene is synthesized in higher vascular plant tissue from methionine by a three-step process (*King & O'Donoghue, 1995*). This biosynthetic pathway is outlined below:

L-methionine → S-adebosyl-L-methionine (AdoMet)

S-adebosyl-L-methionine (AdoMet)→ 1-aminocyclopropane-1-carboxylic acid (ACC)

1-aminocyclopropane-1-carboxylic acid (ACC) → ethylene.

There are two enzymes unique to this pathway, ACC synthase and ACC oxidase. They catalyze the conversion of AdoMet to ACC and ACC to ethylene, respectively (*Mathooko, 1996, 7*).

The synthesis and action of ethylene involves complex metabolic processes, which require oxygen and are sensitive to elevated carbon dioxide. Endogenous sensitivity to ethylene alters during plant development, as does the rate of synthesis and loss by diffusion from the plant (*Saltveit, 1999*).

In a plant system the last step in ethylene production is oxidative and therefore requires adequate oxygen levels within the skin of the tubers. Oxygen penetrating the skin could in extreme conditions be restricted by its 'barrier' nature.

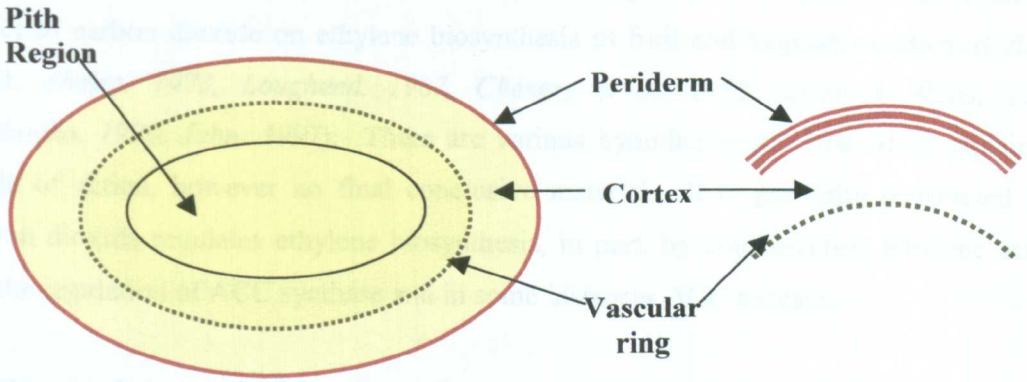


Figure 30 Schematic diagram of a potato tuber cut through its transverse axis. Not to scale. (*MacKinnon, 2000*)

When stores are loaded after harvest the crop temperature is kept higher than normal at ~15°C to allow for suberization. This is the deposition of complex fatty substances present in the wall of cork tissue (periderm) that waterproofs it and makes it resistant to decay. Normally the process is complete within approximately two weeks. After which time the storage temperature is reduced for long-term storage. In the case of processing crop the desired storage temperature is between 8-11°C depending on the cultivar and intended usage period.

Under normal conditions of storage both oxygen and carbon dioxide can move through the peripheral layer of tuber tissue via lenticels (free air spaces in tubers). Therefore it is unlikely that the nature of the skin would restrict the potatoes ability to synthesize ethylene. However most of the available oxygen is consumed for respiration within the peripheral layers, so the rate of ethylene production is naturally low. If oxygen concentrations were to become lower than atmospheric levels (~21%) then availability would be a limiting factor in respiration and ethylene production. (*C. Pidgeon, personal communication*). This is the basis for a lot of the controlled atmosphere systems in use in the fruit and vegetable post-harvest industry (*Kader, 1986, Herregods, 1995, Khanbari & Thompson, 1996, Mathooko, 1996, Coleman & M'Inerney, 1997).*

Equally it can be surmised that a build up of ACC, which would happen even during a brief period of anaerobiosis, would lead to a short burst of ethylene production upon returning to air when the ACC is converted.

Through the years there has been a lot of literature published relating to the regulatory effect of carbon dioxide on ethylene biosynthesis in fruit and vegetables (*Burg & Burg, 1965, Abeles, 1973, Lougheed, 1987, Chevery et al, 1988, Sisler & Wood, 1988, Mathooko, 1996, John, 1997*). There are various hypotheses put forward to explain its mode of action, however no final conclusive material. It is generally considered that carbon dioxide regulates ethylene biosynthesis, in part, by counteracting ethylene action via the regulation of ACC synthase and in some instances ACC oxidase.

Ethylene binds to a part of the plant cells and induces its physiological effects (*Johnson & Ecker, 1998*). The influence of many ethylene analogues has been studied to try and identify and further characterise the binding site of ethylene. Thus far it is thought that binding sites are primarily in the outer layers of the tubers underneath the skin (*M. Tully, Personal communication*) and that a copper cofactor is involved in the process of binding. *Bleeker & Kende, 2000* concluded that the ethylene receptors are negative regulators of ethylene responses, in that when ethylene is present it binds to its receptors inactivating them and the protein, which would normally prevent manifestation of ethylene responses. Investigations into potential ethylene inhibitors or blockers continues. The compound that shows most promise at this point in time is 1-methylcyclopropene (MCP) (*Sisler & Serek, 1997, Jiang et al, 1999, Watkins et al, 2000, Fan & Mattheis, 2000).* MCP is very volatile, binds irreversibly to ethylene receptors and is effective at very low concentration.

It is worth noting that sucrose reserves, which are hydrolysed to glucose and fructose and undergo glycolysis, subsequently provide the carbon skeletons for biosynthetic reactions as described in the previous pages (*King & O'Donoghue, 1995*).

Following on from this literature study of ethylene practical aspects such as the natural level of production and evolution from potato tubers were examined. These experiments are presented in the pages that follow.

5.1.2 Natural levels of ethylene production by potatoes in storage

5.1.2.1 Introduction

Experimental work conducted as part of this project has found that the level of natural ethylene production achieved an atmospheric concentration in the approximate range 0.50-1.5 nanolitres/litre in 24 hours from 2kg of tubers. This internally produced ethylene is in the region of one thousand times less than levels measured in potato stores post fogging (0.2-10µl/L).

This measured concentration equates to a 0.05-0.16nanolitres/kg/hr rate of evolution. Using *Burton & Meigh's, 1971* relationship between the volume and weight of ethylene at 10°C and normal atmospheric pressure (1µl ≈1.2µg) these convert into 0.06-0.19ng/kg/hr.

5.1.2.2 Method

Each treatment was applied to 2kg of potatoes (cultivar Cara) in a 5l desiccator. The items referred to as desiccators are 5l glass storage containers that had the desiccant material removed for this series of experiments. They were pre-treated with a silanizing agent to prevent absorption of ethylene onto the glass.

The desiccators were sealed and placed inside an incubator at the required temperature. Each treatment was carried out in duplicate. Both replicates of each treatment were stored inside the same incubator. Different treatments were kept in separate incubators. A 2ml gas sample was collected from the headspace of each desiccator via a gas-tight syringe and analysed by gas chromatography (GC) for ethylene content. As the samples contained very minute amounts, concentrated headspace gas samples had to taken to ensure detectable and quantifiable results.

Silanised glass tubes (150mm length) containing a 15mm resin bed of Carbosieve SIII (previously conditioned, see chapter 2) were connected onto the rubber bung in the desiccators by silicone tubing, a glass tube (3mm i.d.) inserted through the rubber bung and connected to a wide bore needle (the rubber bungs were covered with teflon tape, PTFE). This allowed 2.5l of headspace gas to be pulled through the resin bed at a rate of 50ml/min by the air sampling pump attached (total sampling time 50 minutes). A length of silicone tubing was attached to the inner side of the glass tube to ensure the air being sampled was representative and not solely from the region around the bung. There was a another wide

bore needle inserted into a second identical glass tube also through the rubber bung to prevent the system from pressurising, this was opened one minute after sampling was initiated and closed when completed. When sampling was finished the resin was removed from the tube and placed into a 5ml high-pressure headspace vial and analysed according to the method described in chapter two.

5.1.2.3 Design

The objective was to determine the production rate of ethylene from potatoes in a steady state. Secondly the aim was to compare the tuber response in terms of increased ethylene synthesis to different stressful situation.

The treatments included stresses such as elevated carbon dioxide levels, elevated storage temperatures and applying external sources of ethylene. They are detailed in the table below.

	Treatment A	Treatment B
Experiment 1	Normal[1]	1.5-2.0% CO_2
Experiment 2	Normal[1]	25°C, 40°C
Experiment 3	Normal[1]	2ppm C_2H_4

Table 29 Summary of environmental conditions used in treatments to study natural ethylene production

Carbon dioxide was applied into the desiccators via the glass tube through the rubber bung. The source was a standard 10% carbon dioxide in air, from a pressurised cylinder. 2l of this was passed into the appropriate desiccators at a rate of 100mls/min. The total application time taken was 20 minutes. This equates to 200,000µl carbon dioxide into 5l, which is an application concentration of 4% carbon dioxide inside each of the desiccators. Some loss is expected because the system has to be open during application to prevent pressure build up, for this reason the concentration was tested to check prior to sampling to determine actual storage conditions. To do this 10ml of headspace gas was extracted using a 10ml disposable plastic syringe. The gas was bubbled through 1ml of 1M NaOH

[1] Normal is 10°C and normal air

solution to trap the carbon dioxide. An appropriate blank was included to account for carbon dioxide in the air. The trapping solution was analysed according to the methods described in chapter two. The concentration of carbon dioxide determined to be present inside the desiccator while in storage was between 1.5-2.0%.

External ethylene was applied from a standard pressurised cylinder of 100ppm in helium. 100mls was extracted from the cylinder and injected into the appropriate desiccators. This equates to 10µl ethylene into a 5l vessel, yielding a concentration of 2ppm. The existence of ethylene in the desiccators was transitory as ethylene diffuses relatively rapidly. Therefore when the 24 h samples were collected there was only negligible amounts of the externally applied ethylene remaining in the desiccator atmosphere.

Each desiccator containing tuber samples was placed inside an incubator at 10°C to settle for three days prior to the initiation of treatments. The lids were placed onto the desiccators immediately before treatments began and the ends of the glass tubes (through the rubber bung) were sealed with Teflon tape (PTFE). Samples were collected 24 and 48 hours after treatments were started, except in the case of the external ethylene treatment because of technical problems with the apparatus. During sampling the incubators were flushed to remove any residual ethylene. After the 24-hour samples were collected the desiccators were left open for 30 minutes inside the incubators to allow any accumulated carbon dioxide to disperse and complete exchange with ambient air within the incubator to occur.

5.1.2.4 Results and Discussion

The ethylene production rates expressed below are the mean values for each treatment. There are no standard deviations shown due to very low values and daily variability. The results are expected only to give an indication of the order of magnitude of naturally evolved ethylene in potatoes and if this is influenced greatly by particular stresses. The units are nl/kg/hr.

Experiment 1	Normal	1.5-2.0% CO_2
24 h	0.101	0.075
48 h	0.087	0.120

Table 30 Tubers in normal storage compared with elevated CO_2 storage conditions

Experiment 2	Normal	25°C	40°C
24 h	0.057	0.070	0.152
48 h	0.069	0.070	0.108

Table 31 Tubers in normal storage compared with elevated temperature conditions

Experiment 3	Normal	2ppm C_2H_4
24 h	0.106	0.070

Table 32 Tubers in normal storage compared with an external source of ethylene

The tubers evolved natural ethylene under normal conditions of storage.

Overall levels detected here were notably lower than those stated by *Burton & Meigh, 1971* (~1ng/kg/hr ≈0.83nl/kg/hr), by a factor of 10 in most instances but up to 15 times less in some cases. This could have been a factor of the sample collection or desorption technique that was not as efficient as hoped. If this were the case all the samples and standards would be biased in the same direction and to the same extent. As calibration for this method was conducted in the same manner as sample handling the comparison between treatments is still valid.

Under conditions of elevated carbon dioxide (~1.5-2%), ethylene production rate was not apparently different to that in normal atmospheric conditions.

The production of ethylene was increased most by elevating the storage temperature from the standard 10°C. The effect was not distinguishable at 25°C, but at 40°C production rate had in most cases approximately doubled.

Application of exogenous ethylene (2ppm) did not in generate a detectable increase in ethylene production. Therefore it is not conclusive from these results that exposure to an external concentration of 2ppm ethylene will yield increased synthesis of endogenous ethylene. It must be borne in mind that the levels discussed are minute amounts and an increase in production over this short time scale may not be enough to alter the quantifiable concentration. There is extensive literature that shows an increase in biosynthesis can be expected when an exogenous source is applied due to the autocatalytic reaction pathway (*Mathooko, 1996, 7*).

The likely sources of environmental stress to which tubers may be subjected while in storage include short-term exposure to higher carbon dioxide concentrations than normal following fogging, warm fog entering stores during sprout suppression treatment and the presence of residual ethylene from the fogging process.

It is reasonable to surmise that as a result of a standard CIPC thermal fog treatment the production of natural ethylene will increase to some degree.

The precise effect of ethylene on fry colour has to be established.

5.1.3 Crop storage trial to determine the effect of ethylene on processing quality

5.1.3.1 Introduction

Experimental work to date has shown that ethylene contamination of CIPC fogs is widespread. Levels of contamination under UK conditions have been typically lower (0.2-10ppm) than those reported in North America of 0-60ppm (*Wang and Pritchard, 1997*). Investigation found that the ethylene identified in stores is largely a result of exhausting the combustion gases of hydrocarbon fuel into the store. The levels detected are known to be physiologically significant to tubers. The objective of this work was to examine and compare the effect on crop of ethylene derived from combustion of fuel and from a standard synthesised source. The source in this case is a generator similar to those used regularly in banana stores to induce ripening.

5.1.3.2 Experimental work

A trial was conducted at SBEU to establish the effect of ethylene (in the concentration range detected in experimental stores) on potato tubers in storage under conditions of short-term exposure. Each stage of the fog generation was tested for its effect on fry colour. These treatments were compared to the effect of applying a standard concentration of gaseous ethylene to crop in storage.

All thermal fog based treatments were petrol fuelled and applied by an experienced applicator using a Stored Crop Conservation Unifog machine. Ethylene was applied using a generator that was placed inside the store. A set volume of denatured ethanol was delivered onto the metal catalyst inside the generator. From this a volume of ethylene gas was evolved which equates to 5-10ppm in the store atmosphere. The volume of alcohol required for this atmospheric concentration is predetermined based on the volume of the store. There was little control over the alcohol solution once it had been transferred quantitatively into the delivery vessel that feeds it into the generator. For this reason uptake efficiency or rate of ethylene production was unknown. Only a small volume was used and the generation of ethylene is thought to be a quick process. It was assumed that the store atmosphere would have reached equilibrium within an hour of starting the application.

5.1.3.3 Method

Approximately 30 tubers (cultivar Saturna) were placed in each 10kg tray. There were 12 trays for each treatment (3 replicates per sampling occasion). The different treatments were carried out in separate stores. The trays were arranged randomly in one layer on top of a wooden pallet inside the 12 tonne stores. Three six tonne boxes were placed underneath the pallets to reduce the air to crop ratio and place the samples at a uniform height above the floor of the store.

In the 24-hour period before ventilation the oxygen, carbon dioxide and ethylene concentrations in the individual store atmospheres were measured. A Hitec landfill gas meter was used for determining oxygen and carbon dioxide concentrations (sensitive down to 0.1%). A semi-quantitative method of ethylene analysis was employed. The colorimetric Gastec system (Anachem no. 172L), as described in chapter 2, gives a reading of the concentration range of ethylene in the sampled air. It is sufficient to illustrate that the compound was present in the spiked stores, and as a contaminant in those treated with the fogger, for the duration of 22 hours.

Twenty-four hours after treatments initiated all samples were moved into the one store, in which untreated crop was held from the start of the trial. This move simulated ventilation. All stores used were held at 10°C and 95% relative humidity.

The fry colour of crisp samples was compared for brightness and % fry defects (commercial acceptability). The sample collection, preparation, procedure and analysis were conducted as per standard operating procedure at SBEU as directed by the BPC. This procedure is detailed in chapter 2.

5.1.3.4 Design

The only treatment that was duplicated was the ethylene spike as this was a new treatment. The others were only carried out on the one occasion for this trial due to restrictions on store availability. Each sample consisted of an entire tray of 30 tubers. Three replicate samples were collected for each treatment per sampling occasion. Samples were collected on days 1, 7, 14 and 28 of storage.

Treatments compared for effect on the processing quality of potatoes				
Untreated	Exhaust only	Exhaust +Methanol	CIPC fog	Ethylene spike (5-10ppm)

Table 33 The treatments compared for effect of ethylene on processing quality

The methanol referred to in the third treatment in *Table 33* above is the equivalent volume of solvent that would normally enter a store during a treatment with a 50% methanol based CIPC formulation. The formulation used for CIPC fog was 50% CIPC in methanol (MSS 50M) from Whytes Agrochemicals. The volume of CIPC formulation applied is calculated based on a full 12 tonne store.

All of the samples were placed in storage three days before treatment started to allow them to settle in the storage conditions.

5.1.3.5 Results and Discussion

The graph overleaf displays the mean Hunter L-value of the 3 replicates of a given treatment. Plus and minus one standard deviation is plotted on each point to indicate the variability. Ethylene spike results are the mean of each replicate of the treatment, thereby each bar encompasses six samples instead of three. On day 0 three lots of 30 tubers that had been stored under the same conditions were removed and processed to give a mean background fry colour representative of the entire crop.

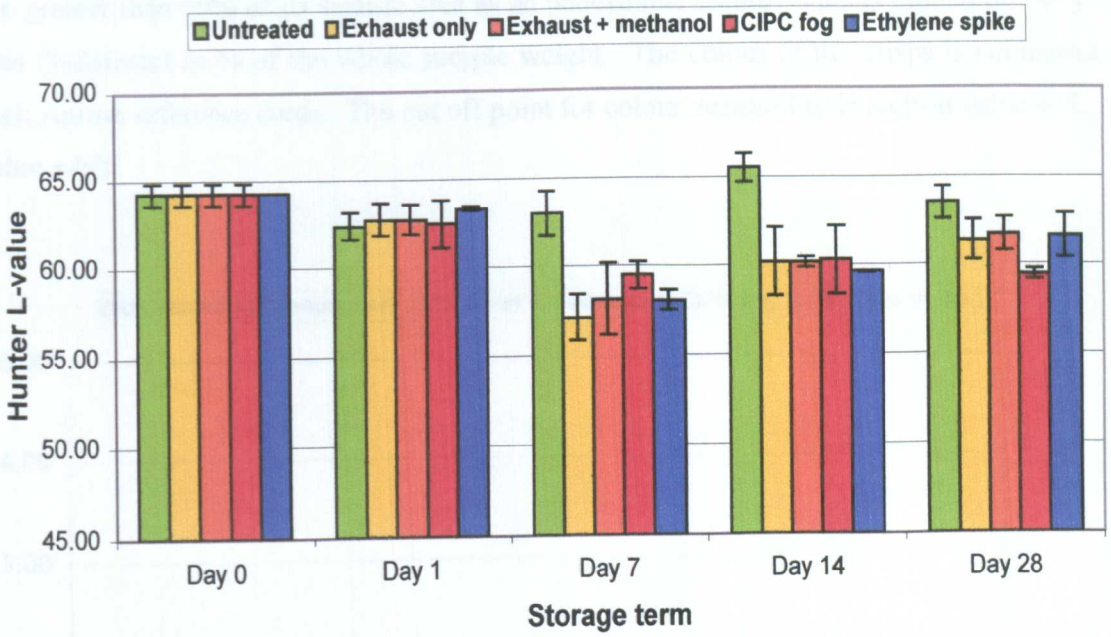


Figure 31 Comparison of the effect of ethylene from different sources on fry colour (n=3)

With the exception of day 1 samples the untreated crop maintained the best fry colour for the 28 days of the trial. All fog treatments resulted in a darker fry colour of the crisp samples. The ethylene spike also resulted in darker fry colours, similar to that of the fog treatments. The crop exposed to fog treatments and ethylene did show an improvement in fry colour toward the 28-day samples, however remained darker than the untreated.

Untreated samples were significantly different to all other treatments on day 7 and 14 only. However by day 28 the untreated was only significantly different to the ethylene treatment.

Treatment	Day 1	Day 7	Day 14	Day 28
Untreated	a	a	a	a
Exhaust	a	b	b	ab
Exhaust + Methanol	a	b	b	ab
Ethylene	a	b	b	ac
CIPC	a	b	b	b

Table 34 Statistical analysis of L-values. Different letters denote significant differences

The defect values plotted on the following graph are considered the most indicative of all measured defects for market acceptability. It is the proportion of the sample of crisps that has greater than 50% of its surface area as an undesirable colour. This is plotted on the y-axis (%defects) as % of the whole sample weight. The colour of the crisps is compared with Agtron reference cards. The cut off point for colour desirability is Agtron value 4 (L-value ≤49).

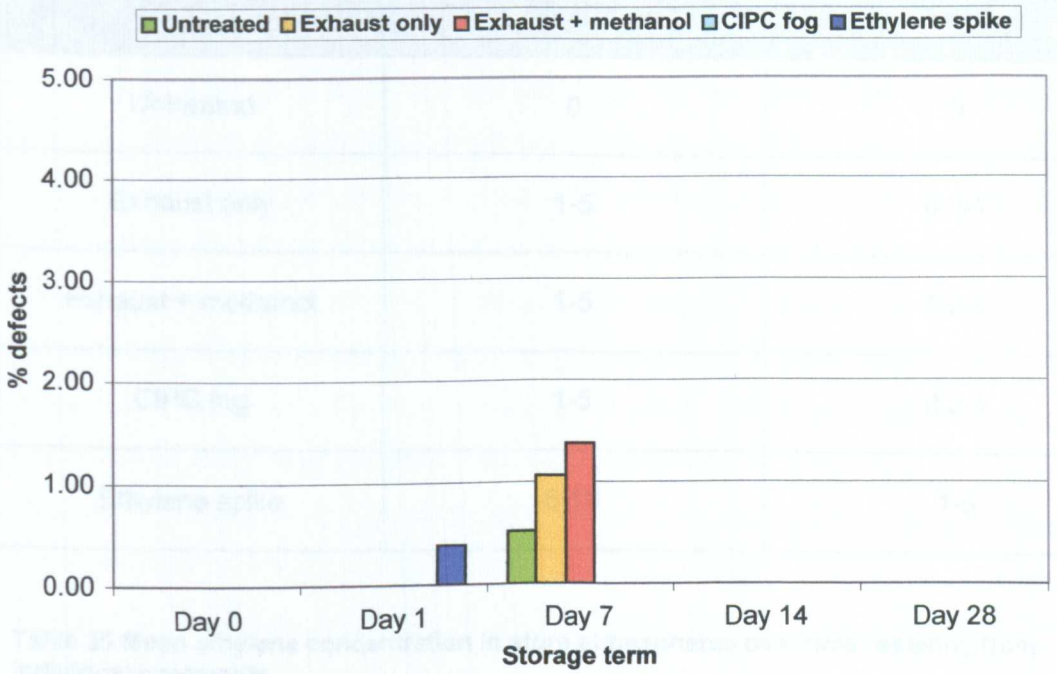


Figure 32 Comparison of the effect of ethylene from different sources on % fry defects

Defects were generally very low for all samples from all treatments. No standard deviations are plotted because most of the samples even those in day 7 had no defects and the mean values plotted only serve to indicate that the effects of ethylene tend to be more pronounced 7 days after exposure.

The atmospheric gas analysis results found that any carbon dioxide present in the stores was below 0.1% and therefore would not affect the processing quality of the tubers. Oxygen was hardly altered from normal atmospheric levels (~21%) by any treatment.

Ethylene was detected in all stores that were treated by the fogger or ethylene generator (*Table 35*). No ethylene was detected in the untreated store. There was some discoloration

in the Gastec samples tubes when used in stores treated with the fogger. The entire reactive bed turned pale grey in colour after sampling, however there was a distinct dark region in all tubes, similar to those without discoloration problems. The values assigned to these treatments were the darkest region of the resin bed. The mean of the values from the ethylene-spiked stores is entered in the table.

Ethylene concentration over time (ppm)		
Treatment	**6 hr**	**22 hr**
Untreated	0	0
Exhaust only	1-5	0.2-1
Exhaust + methanol	1-5	0.2-1
CIPC fog	1-5	0.2-1
Ethylene spike	5-10	1-5

Table 35 Mean ethylene concentration in store atmospheres over time resulting from individual treatments

Ethylene was generated in equal amounts from all stages of the fogging process again concluding that the exhaust gases are the source. The levels in stores treated by the generator were higher for the entire 24 hours until ventilation. The concentration in stores had declined over this but in all stores the gas was still present until deliberate ventilation.

5.1.3.6 Conclusions

Each stage caused a decline in fry colour as they all involve combustion products of petrol. The fry colour graph demonstrates that crop exposed to ethylene (from the generator) suffered a decrease in quality in the same way. All except the untreated samples were subject to the darkening of fry colour that is symptomatic of CIPC thermal fog application.

Headspace samples of commercial storage atmospheres were needed to confirm the presence of ethylene in these type of stores after thermal fog treatment.

5.1.4 Potato store atmosphere samples

5.1.4.1 Introduction

Since the start of the project there have been problems with how to practically determine the ethylene concentration in the store air. In the trials at commercial stores Gastec colorimetric detection tubes have been employed. These provide a quick result for the range of concentration that ethylene is present in. They have been particularly useful as a practical and efficient method of determination throughout the trial. Chapter two shows how well the Gastec results correlate with the GC analysis of ethylene atmospheric samples. However on occasions it would have been more useful to know the concentration in stores to differentiate ethylene production to a more accurate level and therefore differentiate between the outcomes of similar treatments. GC analysis would be an ideal method for doing this. As already discussed in chapter 2, it had not been possible to collect, transport and quantitatively analyse gas samples from store atmospheres while GC equipment remained in a different location to the stores. Methods of collecting headspace samples other than using adsorbent resin were explored and the simplest seemed to be to transport a proportion of the store air as a gas and subsequently sample from this. Providing sample integrity could be retained within a suitable leak-proof container for a number of days (three maximum, to allow transit) this was a feasible option. These experiments were not conducted until later on in the project owing to the workload. Therefore they have only been used in these small one off experiments toward the end of the third storage season.

5.1.4.2 Sample collection and transportation

Originally plastic gas sample bags were considered. There were 2l capacity bags available that were impermeable to ethylene, but had to be filled under vacuum (Alltech 41082). Additional costly equipment would have been necessary to do this.

Secondly 5l bucket style tins (similar to domestic paint tins) were tested. They are lightweight and so although transporting them would be bulky, it would not be particularly expensive.

The open tins were placed inside the stores before thermal fog application. This time allowed the tins to be filled with store air under steady state conditions. The lids were placed on top of the tins immediately the door of the store was opened and the individual entered the store, thereby minimising any dilution effect of exchanging store air with ambient. Septum ports were fitted to the lids of the tins to allow gas samples to be withdrawn on arrival at GU where GC analysis was carried out. The temperature of the tins was kept as constant of possible (approximately 20°C) once removed from the store until prepared for analysis at GU when the temperature was lowered. This was done to simulate store conditions as they were at the time of sample collection at approximately 3°C. P.T.F.E. Teflon tape was used to ensure a completely air-proof seal around the septum ports and also was used to line the open rim at the top of the tin. This made certain that when lids were firmly secured on top and no air could pass through the seal.

As an additional measure two side taps were fitted to each tin with connectors for tubing when required. This way tins could be filled remotely by lowering the tubing in through the roof of the stores and drawing store air through it. These taps were vertically offset so that the gas would not simply be pulled straight out of the container without dispersing inside. So by allowing at least three times the volume of the tins to pass through before closing both taps simultaneously, a representative samples of store air could be collected. The taps used were suitable for high temperature applications in case sample collection during fog application was involved.

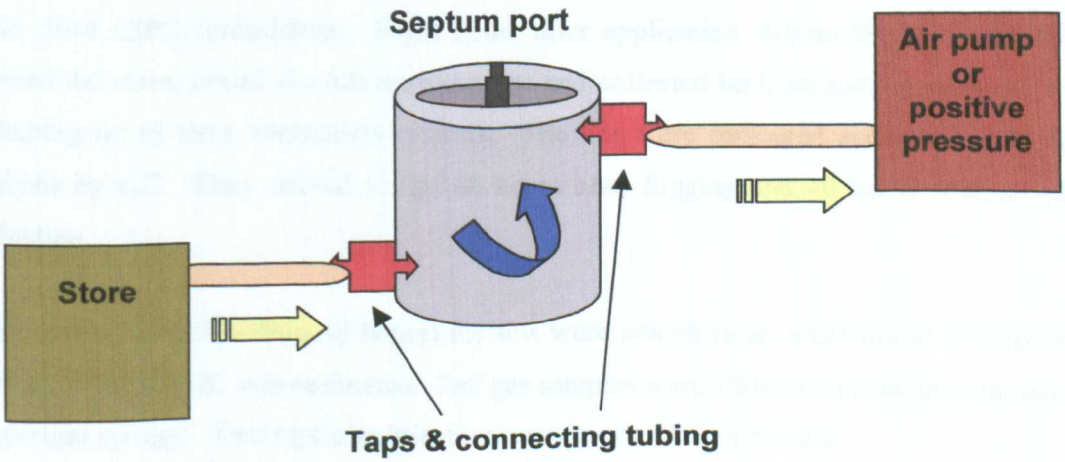


Figure 33 Schematic diagram of 5l tins designed for collecting and transporting store atmosphere samples

Fortunately the basic sampling procedure was satisfactory. Consequently this meant the additional tap system was never fully tested in a real situation.

Testing tins for sample integrity

The 5l tins were tested at GU by spiking the tin atmosphere with specific concentrations of ethylene from pressurised cylinders. The ethylene levels were monitored over time by withdrawing 2ml gas samples via the septum port in the lid using a gas-tight syringe. Ethylene concentration within the range of 2-20ppm can be maintained in the tins at 20°C for up to three days before degradation starts. Once this starts ethylene reduces quickly to below quantifiable levels. As time passed and the ethylene concentration lowered the chromatograms became more complex, with additional peaks present.

5.1.4.3 Sampling from a Commercial potato store

The atmosphere inside a full size commercial store was sampled following thermal fog application to determine precisely the ethylene concentration resulting from fog treatment.

Design and Methods

Two open 5l tins were placed inside the 1500 tonne store before fogging. Stored Crop Conservation Ltd treated the store (crop temperature approximately 3°C) with 63.75l of MSS 50M CIPC formulation. Eight hours after application Adrian Briddon of SBEU entered the store, sealed the lids onto the tins and collected both air samples, prior to the switching on of store ventilation systems. The tins were packaged and sent to GU for analysis by GC. They arrived at GU 48 hours after fogging and 40 hours after sample collection.

Upon arrival at GU (within 48 hours) the tins were placed in an incubator at 3°C for 90 minutes while the GC was calibrated. 2ml gas samples were withdrawn from the tins using a gas-tight syringe. Two replicate injections were made of each sample.

5.1.4.4 Results and Discussion

Ethylene concentration (ppm)	
sample 1	1.69
sample 2	1.57
mean	**1.63**
standard deviation	*0.085*

Table 36 Ethylene concentration in 5l store atmosphere samples collected following fogging

The results of each sample corresponded well and the associated standard deviation is relatively small. The concentration was lower than expected following fogging, however this was the first successful sampling in commercial scale stores. Although the concentration was low it cannot be discounted, as there are no other values for direct comparison from within this project. In the early stages of the project Gastec colorimetric detector tubes were used to sample headspace in a commercial store at Cayton but there was no indication that ethylene was present (these samples were also taken before ventilation at 24 hours post application).

It appears that in larger scale stores ethylene dispersed more readily than in the smaller 12 tonne reasonably leak-proof stores at SBEU. The ethylene resulting from fog application in commercial stores, where some leakage is standard, will remain present until ventilation but is unlikely to persist at high levels. It is surmised from this work that the reason Gastec equipment was unable to detect ethylene in the Cayton stores was that by 24 hours after application the levels had reduced to less than the lower concentration bracket of 0.2-1ppm. Had samples been collected earlier at eight hours after application perhaps detection albeit within the lower concentration bracket would most likely have been possible.

The retention of ethylene in different types of stores will depend on the degree of leakage and the ventilation procedure.

5.1.5 Persistence of ethylene in the store atmosphere

5.1.5.1 Introduction

The persistence of ethylene in store atmospheres is surprising considering its ability to move freely in air and its readiness to absorb onto suitable materials. Semi-commercial trials at SBEU have shown that it can persist in store until ventilation following fog treatment. The current standard period left after fog application before ventilation is twenty-four hours.

Experiments were conducted at GU to determine how much ethylene would be expected to be lost from a storage facility over this 24-hour period. Ethylene can be lost by leakage or dilution via exchange with ambient air as it is very volatile. It can be easily absorbed onto available surfaces and therefore could potentially be lost by absorption onto the walls/floors or boxes within a store. This will vary in individual stores depending on the storage temperature and the degree of relative humidity i.e. if the surfaces are wet. The presence of water can aid the transport of compounds onto and into other materials. In this case ethylene is soluble in water and would more readily be absorbed onto the surface of wood. The extent of leakage and local weather conditions (wind speed, direction and temperature) will influence how much ethylene exits the store prior to ventilation.

5.1.5.2 Experimental work

Two types of storage containers were used to represent the expected loss from potato storage facilities:

The first type was 3.75l plastic tubs with a flat lid that could be completely sealed. These were representative of the SBEU stores. Normally after CIPC has been applied into a store at SBEU it is sealed completely for 24 hours. The leakage in these stores is negligible. To restrict absorption of ethylene onto the plastic the tubs and lids were lined with aluminium foil.

The second type were 18l cardboard boxes with fitted lids. No additional measures were taken to prevent leakage from the seams or corners of the boxes. There was no aluminium foil lining inserted therefore potentially the ethylene could be absorbed onto the walls.

Predominantly stores do not receive ventilation or circulation of air throughout an application of CIPC or for the 24 hours following it. In this situation stores are treated as a closed system until ventilation is started again. However, most if not all, commercial storage facilities have some leakage. The degree of leakage can be related to the type of structure, its age and, among other factors, its use. Thus quantifying this across the board is virtually impossible. It is a feature unique to each store and is influenced by environmental conditions. As such ethylene present from a CIPC application will dissipate and largely be lost from the store within this 24-hour period.

There is no definitive distinction made between absorption onto surfaces or loss by diffusion in this trial. Basically the ethylene remaining over time is viewed as persistence in store atmospheres.

This practical work was concentrated mainly on the persistence of ethylene in a sealed environment (plastic containers).

The emphasis in this work is to examine the worst case scenario; if ethylene was not diluted, leaked out, absorbed onto the walls, floors or boxes. What levels of ethylene could be expected to linger in the atmosphere if the storage facilities were leak-proof?

CIPC losses from potato stores impact on the efficacy and duration of sprout suppression obtained from each treatment. The trend in storage facilities now is to reduce leakage in an attempt to retain as much applied CIPC as possible, particularly with the first application of the season. Leakage and principally application techniques and procedures can bias the distribution of CIPC within a store. Research into this area has suggested that reducing leakage would aid the longevity of each CIPC thermal fog application.

Method

Five tubers, cultivar Saturna, were placed inside each container (except blanks which remained empty) and allowed to settle in the storage conditions selected for the trial for three days prior to treatment.

The ethylene applied to the containers was from standard pressurised cylinders of pure ethylene (1000000ppm) or 100ppm in helium. An appropriate volume was injected through a wall of each container using a gas-tight syringe. The puncture was then covered over with PTFE tape to stop exchange with ambient air. The volume injected was

deliberately kept low to prevent a build up of pressure inside the containers, which could influence leakage. With the exception of temporary needle punctures all the containers were kept closed until the end of the designated trial time.

The containers were kept in controlled temperature storage for either 8 or 24 hours following application. Over this time the headspace inside the containers was sampled and analysed for ethylene content. Samples were collected in a 2ml gas tight syringe. The needle was sealed with a silicone septum for the time taken to transport it to the GC for analysis (approximately 1 minute). The entire gas sample was injected directly onto the top of the column and the concentration of the analyte calculated from the trace produced on the integrator. A calibration graph was produced daily for GC analyses of ethylene because of daily variability attributed to fluctuations in ambient temperature and equipment sensitivity.

Before the trial was undertaken the loss of sample (ethylene) from the 2ml gas-tight syringe while sealed with the silicon septum was determined. This was assessed using ethylene withdrawn directly from the pressurised cylinders. Concentrations in the range of interest (0.5-20.0ppm) were monitored over time, at 1 or 5 minute intervals up to 1 hour. It was shown that even at low concentrations of ethylene loss from the syringe was insignificant. Therefore a short residence time in the barrel with the needle sealed using a silicon septum was a suitable method of transporting gas samples from the temperature controlled storage area to the GC equipment.

Design

Only one variable was tested at a time, i.e. one concentration for a specified period of time. For each treatment in the plastic containers there were 3 replicates and 2 blanks. One blank had five tubers inside and no ethylene applied. This was to account for any background ethylene evolved from the tubers, although it was not expected to be measurable under these conditions. The second blank had no tubers inside but had ethylene applied. It should allow for any interactions between the tubers and the ethylene to become obvious by comparison with this blank. Potential interaction would be absorption of applied ethylene onto the surface of the tubers.

The treatments in the box were not replicated and were not included in the latter experiments due to restrictions in crop availability. The treatments are detailed in full in the table below.

Treatment	8 hours storage	24 hours storage
0.5	✓	✓
1.0	✓	✓
5.0	✓	✓
10.0	✓	✓
20.0	✓	✓

Table 37 Treatments conducted in trial investigating the persistence of ethylene in store atmospheres.

Results and Discussion

These graphs show the mean percent of the total ethylene applied that was still present in the storage container at the time of sampling. This allows the comparison of treatments of different concentrations as the lines are essentially the rate of loss of ethylene from the containers. The mean of 3 replicates is plotted for each treatment. The first set of graphs are for a storage time of 8 hours and the second set are for 24 hours.

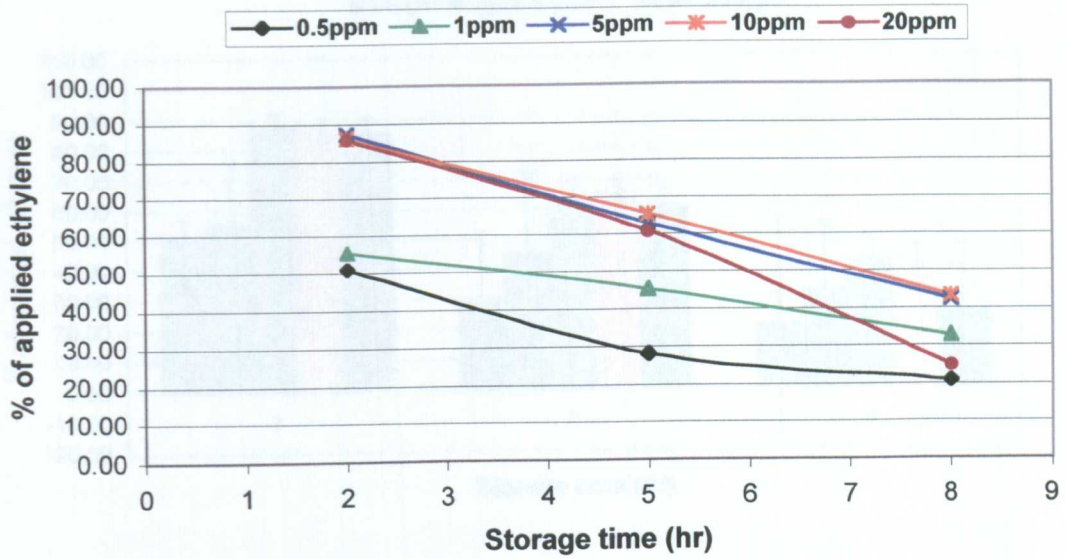


Figure 34 Mean % of applied ethylene present in atmosphere over an 8 hour period

All concentrations still had some ethylene present after 8 hours (20≥%), even at the lower end of range. The 0.5ppm treatment was lost from the containers at the greatest rate, followed by 1ppm. Diffusion of a lower concentration (less source material) will happen over a shorter time scale.

The percentage retained in the first 5 hours was similar for 5, 10 and 20ppm. However the higher concentration showed the largest decline in retention between 5 and 8 hours. This trend was consistent between replicates.

What follows is the same data presented as a bar graph with the associated standard deviations (plus and minus 1 St dev) to allow the variability to be viewed relative to the other treatments.

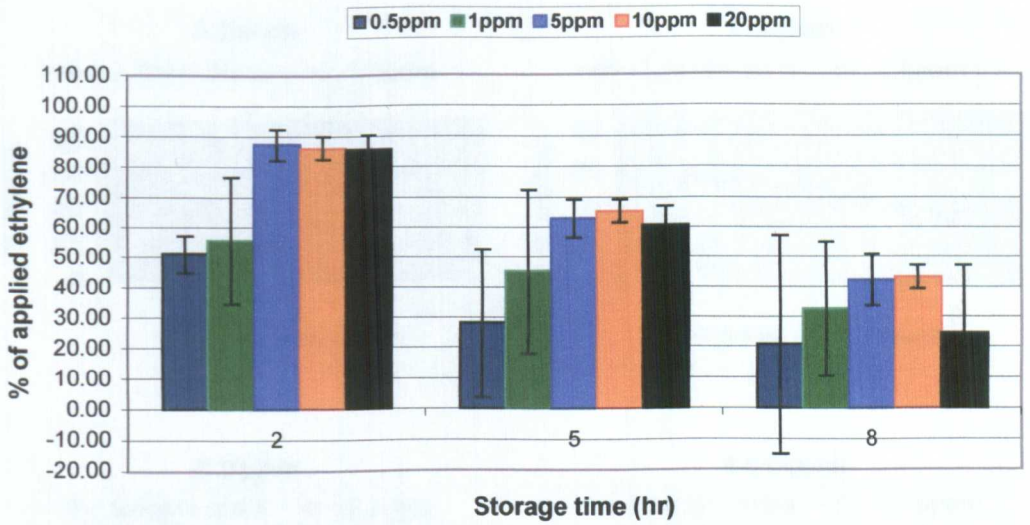


Figure 35 Mean % of applied ethylene present over 8 hours including standard deviations (n=3)

The largest standard deviations are associated with the lower concentrations of treatments and the lower measured values from GC analysis, with the exception of the 8-hour sample of the 20ppm treatment.

This pattern of errors is normal as the lower the sample concentration the greater the error associated with quantifying it. The limit of quantification for this kind of analysis is 0.25ppm from a 2ml gas sample.

The following graphs are a plot of the blank (ethylene applied but no tubers present) for each treatment compared to the treatment mean over time (8 hour trial).

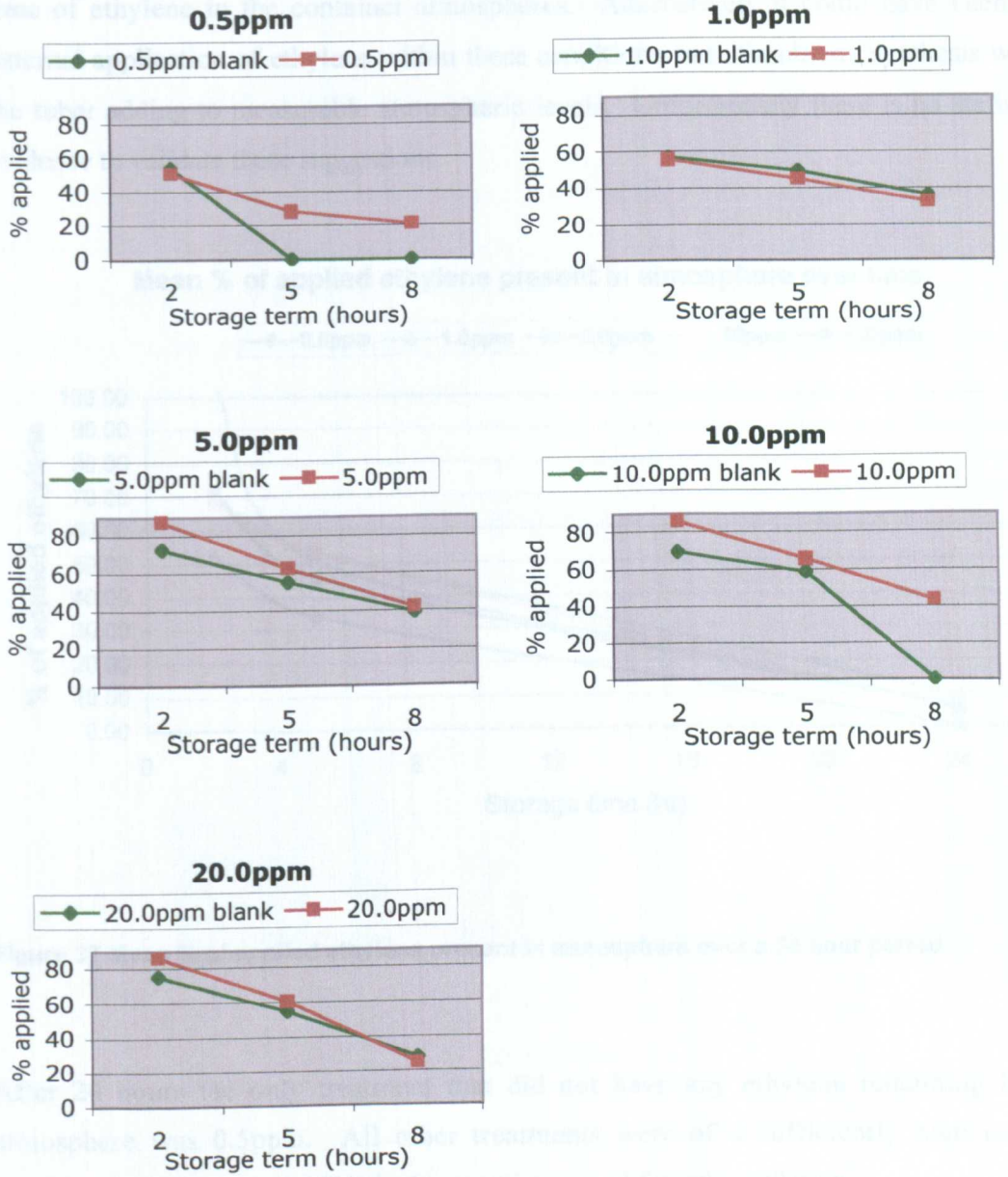


Figure 36 Comparison of the persistence of ethylene over 8 hours with and without the presence of tubers.

The green lines are the blank treatments. These containers had ethylene applied but no tubers inside them. The brown lines represent when ethylene was applied and tubers were present.

With the exception of the 1.0ppm, all the blank treatments dissipated more rapidly inside the 8 hours. It may be possible that the presence of tubers was prolonging the residence time of ethylene in the container atmospheres. Alternatively it could have been that external application of ethylene within these conditions was stimulating synthesis within the tuber adding to measurable atmospheric levels. Unfortunately there is no statistical evidence to validate these suggestions.

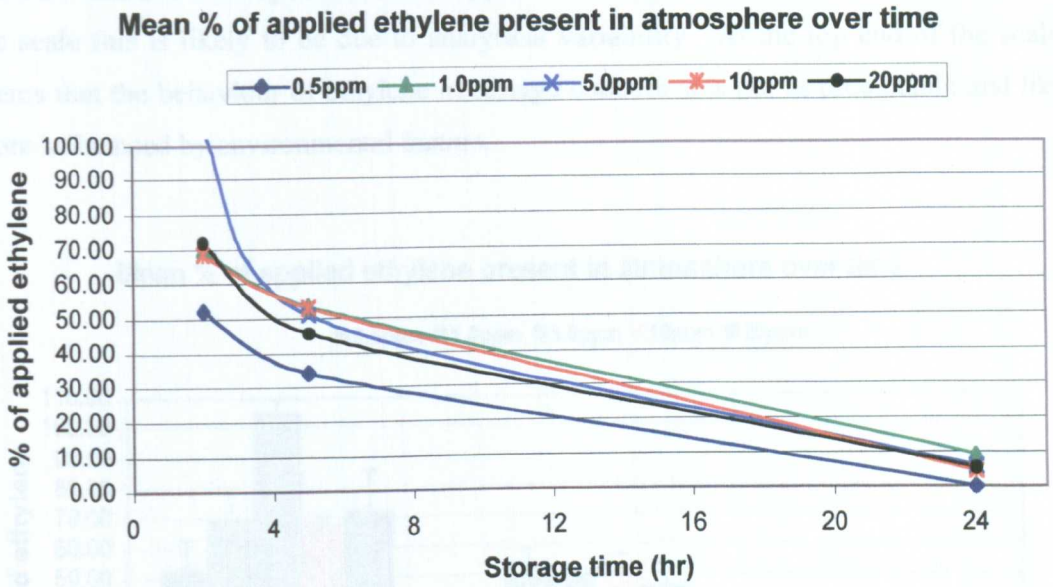


Figure 37 Mean % of applied ethylene present in atmosphere over a 24 hour period

After 24 hours the only treatment that did not have any ethylene remaining in the atmosphere was 0.5ppm. All other treatments were of a sufficiently high enough concentration to persist (≥10%) in the containers until forced ventilation.

As with the 8 hour trial the fastest rate of loss was found to be from the lowest concentration applied. Although overall the 20ppm treatment displayed the second fastest rate of loss in this time scale.

1ppm and 10ppm treatments had virtually equal rates of loss initially then between 5 and 24 hours the rate of the 10ppm treatment exceeded that of the 1ppm. With the 5ppm treatment there appeared to be a lag period before any ethylene was lost. After this time it behaved as the 1 and 10ppm treatments.

The 5ppm treatment sampled at 2hours has a concentration in excess of 100% of the ethylene that had been applied. This could have potentially have been due to stimulated internal levels that were yielding increased measurable levels.

Below is the same data presented as a bar graph with the associated standard deviations.

The standard deviations associated with the mean values in this 24-hour trial are generally smaller, with a few exceptions in the 0.5ppm and 20.0ppm treatments. At the lower end of the scale this is likely to be due to analytical variability. At the top end of the scale it seems that the behaviour of ethylene in storage containers is not as predictable and likely more influenced by environmental factors.

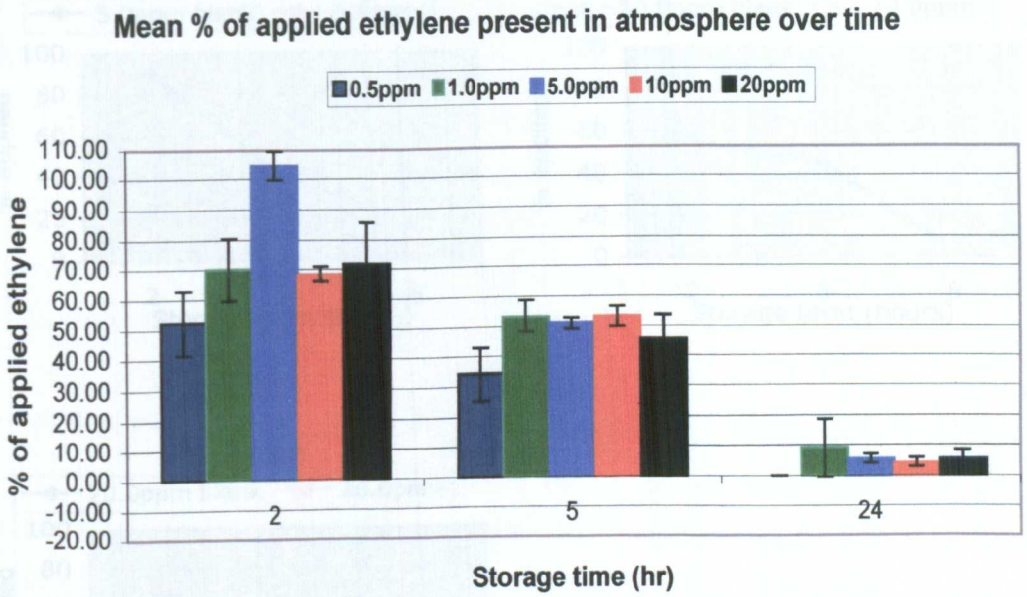


Figure 38 Mean % of ethylene applied present over 24 hours including standard deviations (n=3)

The following graphs are a plot of the blank (ethylene applied but no tubers present) for each treatment compared to the treatment mean over time (8 hour trial). The y-axis is the percent of applied ethylene present in the sampled atmosphere.

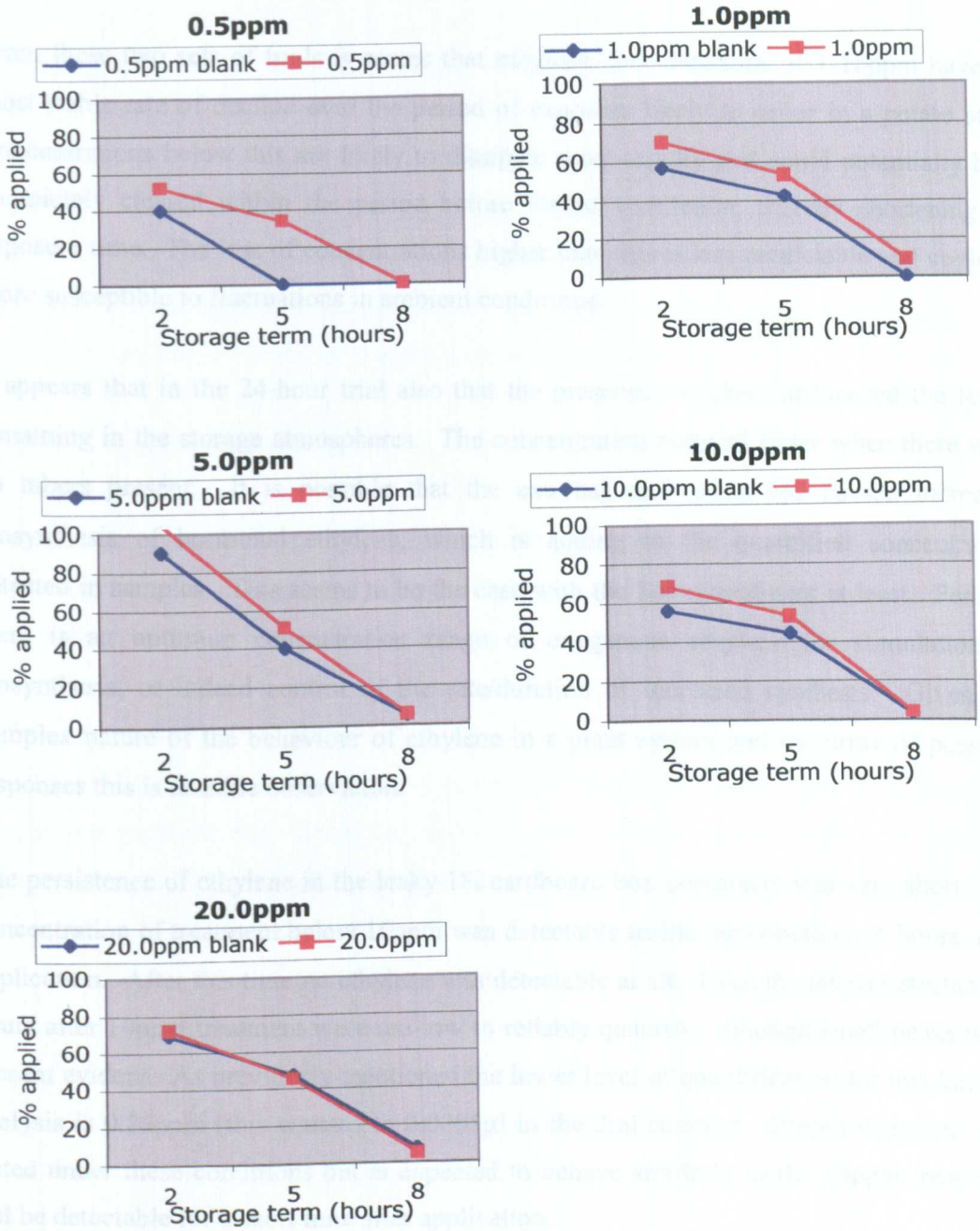


Figure 39 Comparison of the persistence of ethylene over 24 hours with and without the presence of tubers.

The blue lines are the blank treatments. These containers had ethylene applied but no tubers inside them. The red lines represent where ethylene was applied and the tubers were present.

Generally the 8 and 24-hour graphs are comparable for rate of loss within the first 8 hours after application.

From these two sets of trials is seems that ethylene concentrations of 1-10ppm have the most stable rate of decline over the period of exposure likely to occur in a potato store. Concentrations below this are likely to dissipate more rapidly and could potentially have completely cleared within the period before forced ventilation, thereby shortening the exposure time. The loss of concentrations higher than this is less predictable and could be more susceptible to fluctuations in ambient conditions.

It appears that in the 24-hour trial also that the presence of tubers influenced the levels remaining in the storage atmospheres. The concentration reduced faster when there were no tubers present. It is possible that the external application has caused increased biosynthesis of hormonal ethylene, which is adding to the quantified concentration detected in samples. This seems to be the case with the 5ppm treatment at least. Perhaps there is an optimum concentration range of exogenous ethylene for stimulation of biosynthesis, or indeed control of the rate/duration of increased synthesis. Given the complex nature of the behaviour of ethylene in a plant system and the array of possible responses this is feasible observation.

The persistence of ethylene in the leaky 18l cardboard box containers was very short. No concentration of treatment below 10ppm was detectable inside the containers 4 hours after application. After this time no ethylene was detectable at all. Even the levels detectable 4 hours after 10ppm treatment were too low to reliably quantify, although small peaks were present evident. As previously mentioned the lower level of quantification for this kind of analysis is 0.25ppm (this equates to 0.0005µl in the 2ml sample). 20ppm treatment was tested under these conditions but is expected to behave similarly to the 10ppm treatment and be detectable for a short time after application.

Therefore in a particularly 'leaky' store the presence of ethylene (in the range >~5ppm) following fog treatment will be relatively transient. However any higher levels are likely to result in more prolonged exposure but not for the full 24 hours until forced ventilation is applied.

What remains is to focus more specifically on the effects of ethylene alone, not as a constituent of fog, on potatoes in storage.

5.1.6 The effect of exposure to ethylene with varying concentration and time

5.1.6.1 Introduction

Small-scale experiments at GU have found that even low concentrations of ethylene (~ 1ppm) can remain present in closed (simulated) store atmospheres for up to twenty-four hours. In a representative 'leaky' store (considered to be the normal) where some unintentional exchange with ambient air is expected the concentration of ethylene in store will reduce noticeably faster via dispersion into the fresh air.

5.1.6.2 Experimental work

An experiment was conducted in which potatoes in storage were exposed to a range of ethylene concentrations (0, 0.5, 1, 5, 10 & 20ppm). This was carried out for samples in 18l boxes that received deliberate ventilation at 8 or 24 hours after treatment.

The aim of the trial was to monitor the effect on processing quality (fry colour) of potatoes in storage after they had been exposed to different concentrations of ethylene. Secondly the normal exposure time would be dictated by the persistence of ethylene in the store atmosphere and the time period until ventilation after fog treatment. In this experiment the concentration to which the potatoes were exposed could be controlled but the only control over the exposure time was by altering when boxes were deliberately ventilated. As the boxes used were identical and stored within the one communal temperature controlled room, the exchange with ambient air was considered to be equal except for the influence of concentration on the diffusion rate of ethylene. Every day the air within the temperature controlled room was flushed to remove any residual ethylene.

Method

Approximately 60 tubers, cultivar Saturna, were placed inside each box and allowed to settle in the storage conditions for three days before the trial started. No lining was inserted into the cardboard boxes. So potentially the ethylene could be absorbed onto the container walls, however because of the 'leaky' nature of the boxes it was expected that the ethylene would most likely be lost into the ambient atmosphere.

Ethylene was applied into the boxes from pressurised cylinders of standard gas mixtures (100ppm or 10ppm in helium). An appropriate volume was injected through the wall of each box using a gas-tight syringe. The volume injected was maintained as low as possible even for the higher concentrations to prevent forcing exchange with ambient air resulting from application technique. The boxes were kept closed until deliberate ventilation either 8 or 24 hours post application. At the specified time of ventilation boxes were opened for approximately 30minutes to allow several complete air exchanges with ambient and remove any remaining ethylene from the box atmosphere.

The respiration rate of tubers was measured over the storage term. Brown glass jars containing 50ml 1M NaOH to trap the carbon dioxide were placed inside the boxes. After seven days these jars were removed from the boxes and sealed. Another jar with a fresh trapping solution was placed inside each box. These samples were titrated against 1M HCl as described in chapter two.

The processing quality of the potatoes was determined by measuring the fry colour of crisp samples. Ten tubers were collected from each box on each sampling occasion (immediately after the respiration traps were refreshed). A representative sub-sample of these tubers was processed into crisps at GU. The BPC standard operating procedure for crisping was adhered to as closely as possibly. The only modification occurred in sample weights. At GU a single sample originated from 10 tubers, at SBEU/BPC a single sample originated from ~30 tubers. For details of this method and the analysis conducted see *Materials & Methods* chapter. The samples were taken to SBEU for analysis of fry colour and fry defects.

On day 28 at the end of the trial sprouting was measured for those tubers remaining in the boxes (~10). The length of the longest sprout was recorded and the mean length of the longest sprout on each tuber was calculated for each treatment.

Design

Potatoes were stored at 10°C (crop temperature) for 28 days. All boxes were kept in the same temperature controlled-room, therefore ambient conditions were the same for each treatment. Each treatment had three replicates. The control was potatoes not exposed to ethylene, but held under the same conditions. Blanks were included for the determination of respiration rates and subtracted from them to account for background levels.

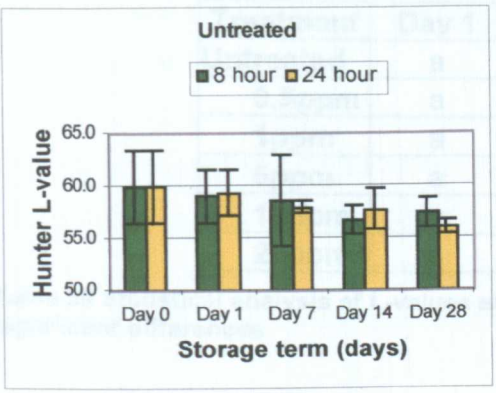
As there were 12 treatments (detailed in *Table 38)* and 3 replicates per treatment, on each sampling occasion 36 samples were collected each for respiration rate and processing quality. There were 5 sampling occasions: Days 1, 7, 14, 21 and 28. A baseline level of fry colour was determined on day 0 previous to ethylene applications by collecting 3 sets of ten tubers from the bulk lot.

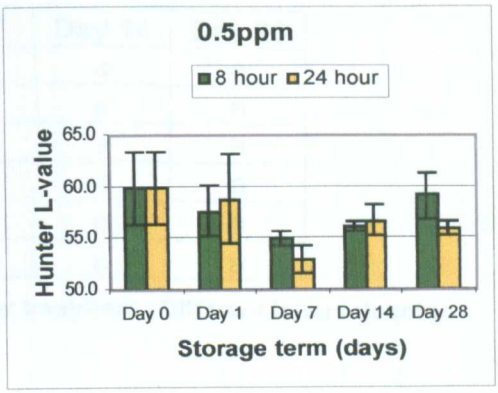
Treatment	8 hours storage	24 hours storage
0.5	✓	✓
1.0	✓	✓
5.0	✓	✓
10.0	✓	✓
20.0	✓	✓

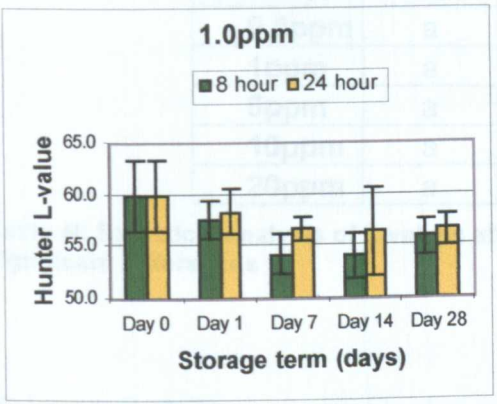
Table 38 Treatments conducted in trial investigating the effect of exposure of ethylene with varying concentration and time.

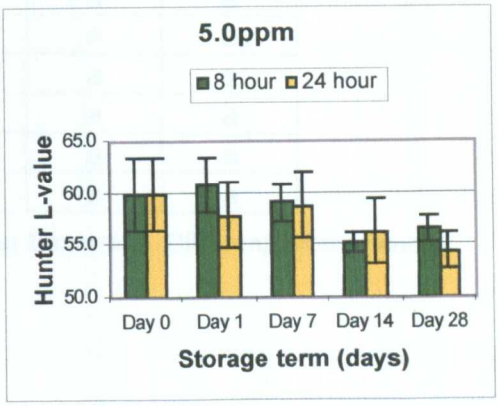
Results and Discussion

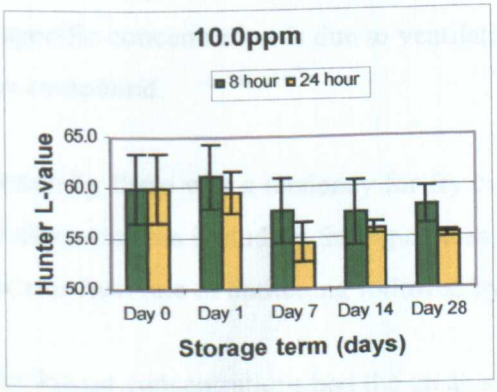
The fry colour results are presented both as individual treatments over time and all treatments compared on the one graph for each sampling occasion. The Hunter L-value is plotted on the y-axis. The higher the number the lighter the fry colour and hence the better the processing quality. The mean result of each treatment is plotted. The mean includes the three replicate samples for each treatment. Each sample consisted of a representative subsample taken from ten tubers from the specific box. Error bars are included on each data point, these represent plus and minus one standard deviation. The first set of graphs is the clearest for illustrating the effect of an individual concentration of ethylene over the period of the experiment.











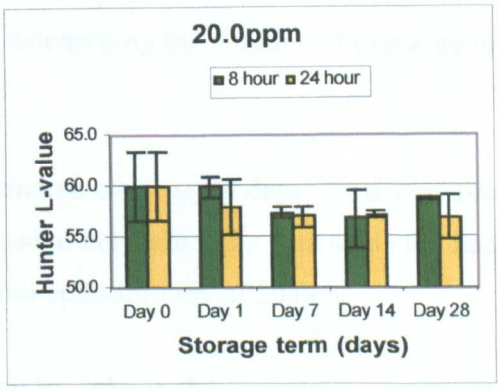


Figure 40 Comparison of the effect on fry colour by different concentrations and exposure times of potatoes to ethylene (n=3)

Treatment	Day 1	Day 7	Day 14	Day 28
Untreated	a	a	a	a
0.5ppm	a	a	a	a
1ppm	a	a	a	a
5ppm	a	a	a	a
10ppm	a	a	a	a
20ppm	a	a	a	a

Table 39 Statistical analysis of L-values after 8 hour treatment. Different letters denote significant differences

Treatment	Day 1	Day 7	Day 14	Day 28
Untreated	a	ab	a	a
0.5ppm	a	c	a	a
1ppm	a	bd	a	a
5ppm	a	b	a	a
10ppm	a	acd	a	a
20ppm	a	bd	a	a

Table 40 Statistical analysis of L-values after 24 hour treatment. Different letters denote significant differences

The potatoes that were not exposed to ethylene had marginally better fry colours overall. There was no distinction between the effect of 8 and 24-hour ventilation times in the untreated crop. Therefore any difference in fry colour of crop treated with ethylene within a specific concentration is due to ventilation times determining the duration of exposure to the compound.

Generally there was a tendency for fry colours to darken over the 28 days. This was true of all treatments including those potatoes not exposed to ethylene. The untreated crop had the steadiest rate of darkening followed by the highest concentration of 20ppm.

The lowest concentrations had the greatest impact on fry colours this was most pronounced between days 1 and 14 of storage. With the 0.5ppm treatment the lower of the L-values altered between 8 and 24-hour exposure over the entire storage time. Neither ventilation time could be clearly identified as the most detrimental to the crop at this concentration. Although not significantly different the mean from the 8hr vent treatment was consistently lower than the mean from the 24hr vent treatment. At these low concentrations the outcome of exposure to ethylene is less predictable even when the exposure time is controlled. There was some recovery in fry colour after day 14 samples suggesting the

effect of short term exposure is transient, but would need approximately a four week period to allow any amelioration.

The effect of exposure to 5.0ppm was similar for both ventilation times and like the 10.0ppm and untreated samples there was a constant decline in the brightness of the crisps at each sampling occasion. However after four weeks the 24-hour exposure was definitely more deleterious at a concentration of 10.0ppm as opposed to the shorter 8-hour ventilation time.

There was a small increase in the mean L-value on the 28-day samples of the 20.0ppm treatment, but this was not substantial. These samples were inclined to follow the same pattern as the upper range of the concentrations tested (5.0ppm plus) that displayed a downward trend in fry colour towards day 28.

Exposure to ethylene resulted in marginally darker fry colour than those of the untreated samples, but there was not a significant difference when the data was analysed.

The following graphs contain the same information however it is presented in a format that allows easier comparison of differences in crop response if at all between treatments and ventilation time.

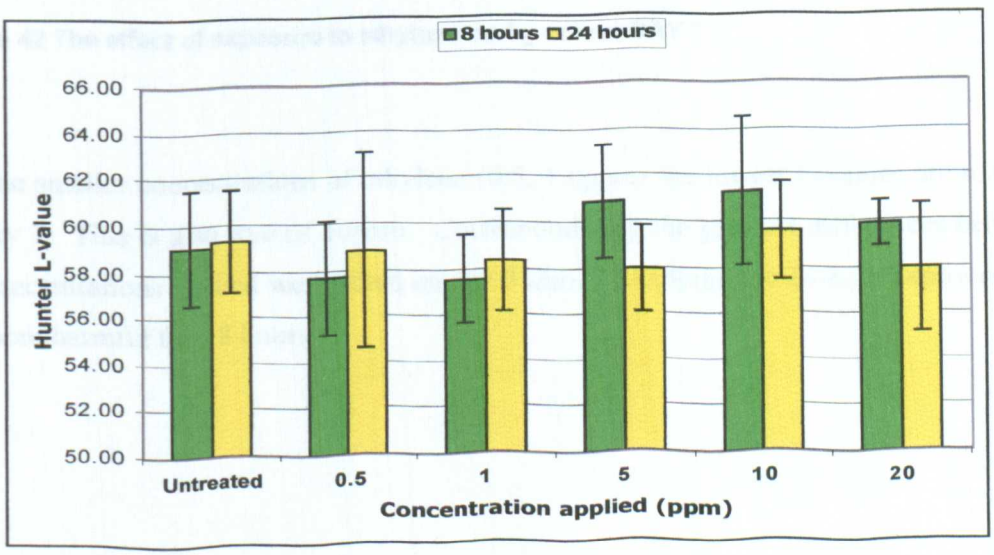


Figure 41 The effect of exposure to ethylene on fry colour-DAY 1

In general the variability between replicates of treatments was quite large. Although it only spans, at most, 9 L-value units on a scale which runs from ~45-75, each unit is vital. Commonly the commercially acceptable L-value for crisp samples is 58 and above. So variability around this level even by a few units is especially important, as it could be the deciding factor for ultimate crop use and sale value.

Due to the variability very few effects could be differentiated statistically with 95% confidence.

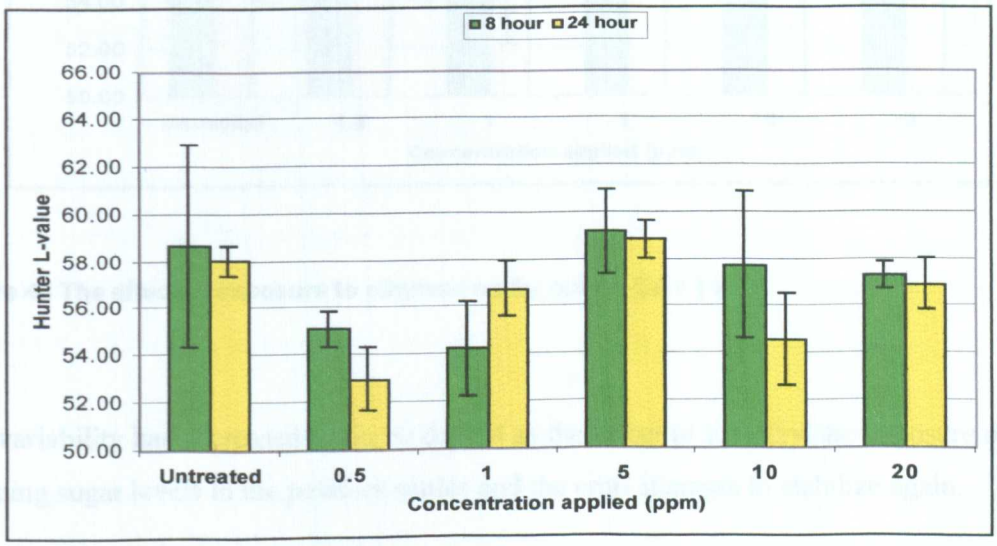


Figure 42 The effect of exposure to ethylene on fry colour-DAY 7

For the smaller concentrations of ethylene (0.5, 1.0ppm) the lowest L-values are reached on day 7. This is also true of 10ppm. Correspondingly the greatest differences between the concentrations applied were noted on day 7 also. At 0.5ppm the 24-hour exposure was the more harmful than 8 hours.

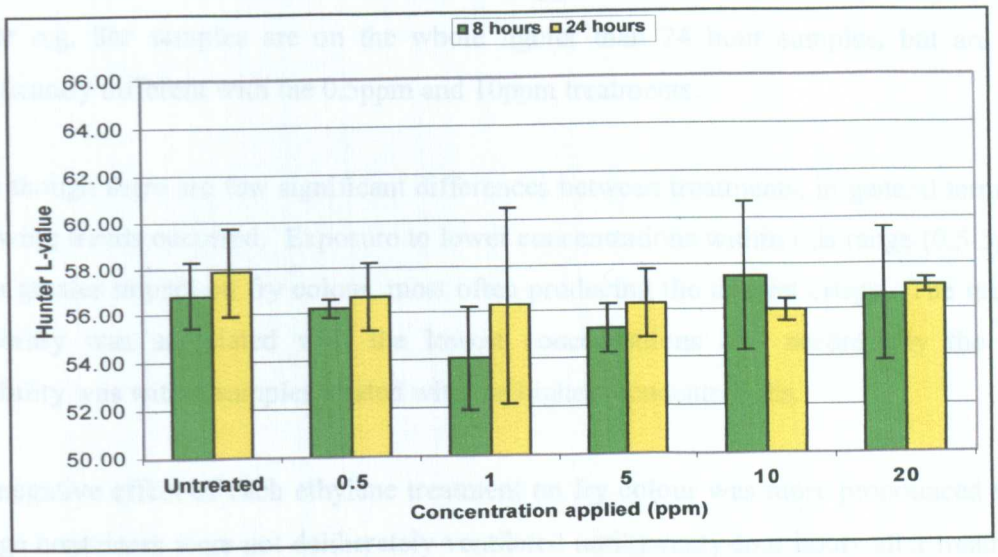


Figure 43 The effect of exposure to ethylene on fry colour-DAY 14

The variability had increased again by day 14 as the effect of the ethylene exposure on the reducing sugar levels in the potatoes settles and the crop attempts to stabilize again.

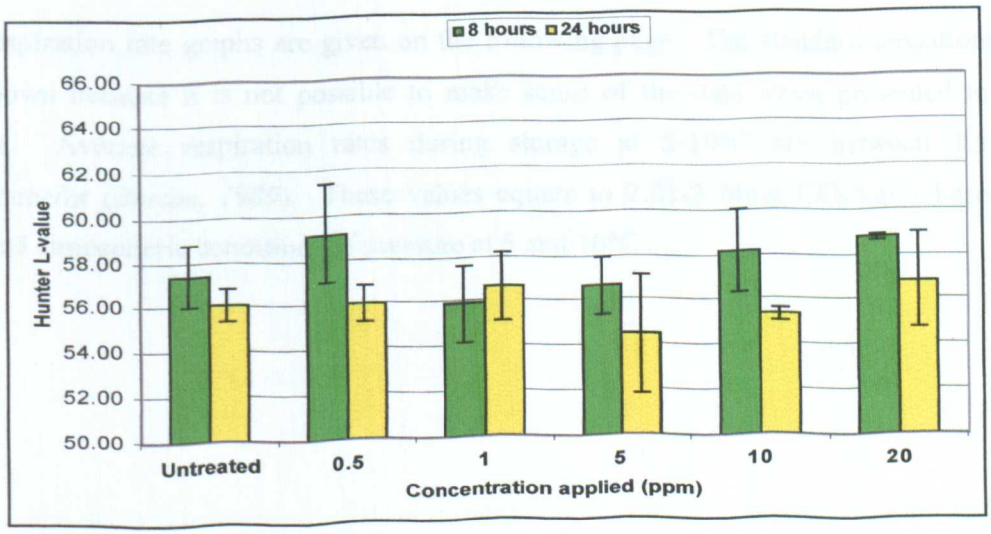


Figure 44 The effect of exposure to ethylene on fry colour-DAY-28

In this last period of storage the effect of not refreshing the air soon enough after treatment to prevent carbon dioxide accumulation or oxygen depletion caused fry colours to be darker e.g. 8hr samples are on the whole lighter than 24 hour samples, but are only significantly different with the 0.5ppm and 10ppm treatments.

Even though there are few significant differences between treatments, in general terms the following trends occurred. Exposure to lower concentrations within this range (0.5-5ppm) had a greater impact on fry colour, most often producing the darkest crisps. The greatest variability was associated with the lowest concentrations and accordingly the least variability was within samples treated with the highest concentrations.

The negative effect of each ethylene treatment on fry colour was more pronounced when storage containers were not deliberately ventilated until twenty-four hours after treatment. Although overall the 24hr exposure resulted in poorer quality the effect was more stable on fry colour. By comparison the 8hr exposure caused greater differences between treatments. There may be an interaction between concentration and exposure time.

The effect of ethylene exposure is negative. It leads to fluctuations in reducing sugar levels in potatoes that encourages aging of tubers. Even though by day 28 fry colours are quite similar among all treatments, the tubers that have been exposed to ethylene particularly at low concentrations will be physiologically older.

The respiration rate graphs are given on the following page. The standard deviations are not shown because it is not possible to make sense of the data when presented in this format. Average respiration rates during storage at 5-10°C are between 1.5-2.0l CO_2/tonne/hr (*Burton, 1989*). These values equate to 2.81-3.74mg CO_2/kg/hr based on standard atmospheric conditions of pressure at 5 and 10°C.

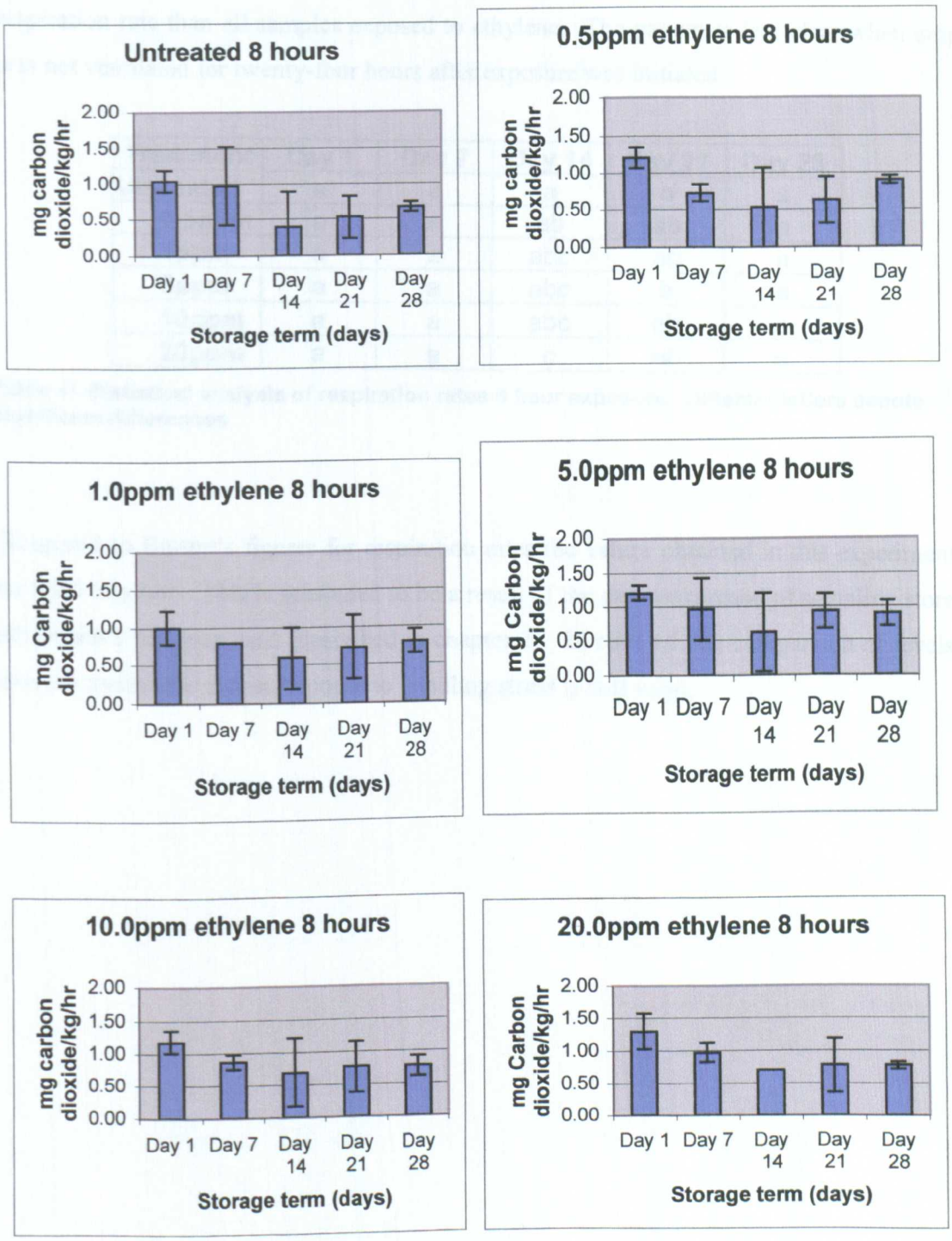


Figure 45 Comparison of the effect on potato respiration rates by exposure to ethylene for 8 hours at different concentrations (n=3)

After a week of storage the respiration rate decreased in samples from all treatments. In containers ventilated eight hours after application the control consistently had a lower respiration rate than all samples exposed to ethylene. The pattern is less clear when crop was not ventilated for twenty-four hours after exposure was initiated.

Treatment	Day 1	Day 7	Day 14	Day 21	Day 28
Untreated	a	a	a	a	a
0.5ppm	a	a	ab	ab	a
1ppm	a	a	abc	ab	a
5ppm	a	a	abc	b	a
10ppm	a	a	abc	ab	a
20ppm	a	a	c	ab	a

Table 41 Statistical analysis of respiration rates-8 hour exposure. Different letters denote significant differences

Compared to Burton's figures for respiration rates the values obtained in this experiment are relatively low. This is presumed to be a result of the passive method of sampling store atmospheres that was used (described in chapter 2). In spite of this comparison of levels between treatments and in response to handling stress is still valid.

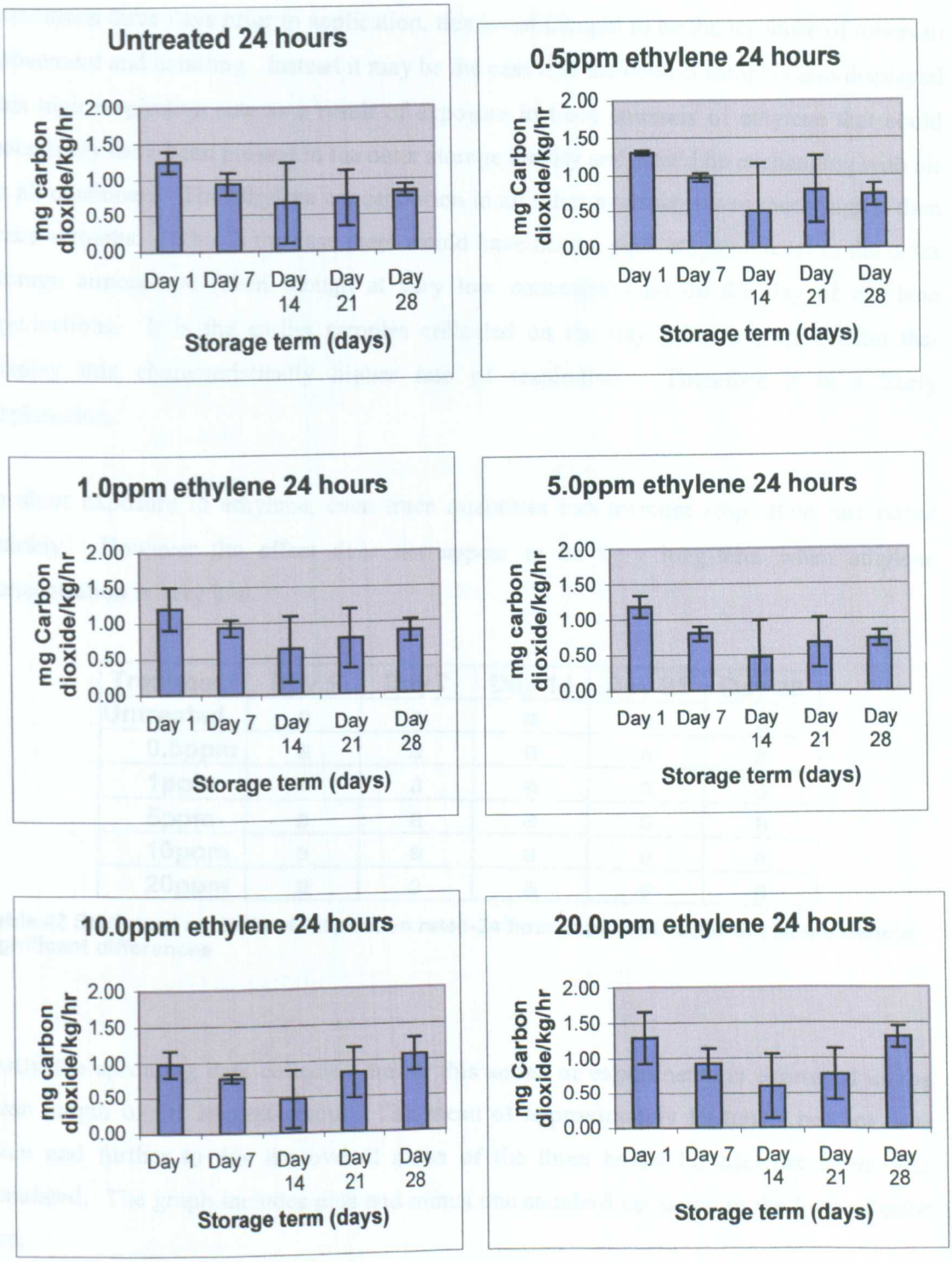


Figure 46 Comparison of the effect on potato respiration rates by exposure to ethylene for 24 hours at different concentrations (n=3)

Respiration rates were high initially in samples from all treatments including the control (no ethylene exposure). As the containers and crop were left to settle in experimental conditions three days prior to application, this is not thought to be the response of tubers to movement and handling. Instead it may be the case that the control samples also displayed this high respiration rate as a result of exposure to trace amounts of ethylene that could potentially have been present in the outer storage facility and would be exchanging with air in all containers. The ethylene concentration in all other treatments was much higher than trace amounts. If this is the case there would have been a peak ethylene level in the outer storage atmosphere (even though at very low concentrations) on the day of ethylene applications. It is the earlier samples collected on the day following application that display this characteristically higher rate of respiration. Therefore it is a likely explanation.

In short exposure to ethylene, even trace quantities can increase respiration rate rather quickly. However the effect does not appear to be very long-term when ethylene concentration is very low.

Treatment	Day 1	Day 7	Day 14	Day 21	Day 28
Untreated	a	a	a	a	a
0.5ppm	a	a	a	a	a
1ppm	a	a	a	a	a
5ppm	a	a	a	a	a
10ppm	a	a	a	a	a
20ppm	a	a	a	a	a

Table 42 Statistical analysis of respiration rates-24 hour exposure. Different letters denote significant differences

Lastly the sprouting data collected during this series of experiments is expressed as the mean length of the longest sprout. The mean of approximately 10 tubers per box was taken and further to this the overall mean of the three boxes for each treatment was calculated. The graph includes plus and minus one standard deviation in the form of error bars.

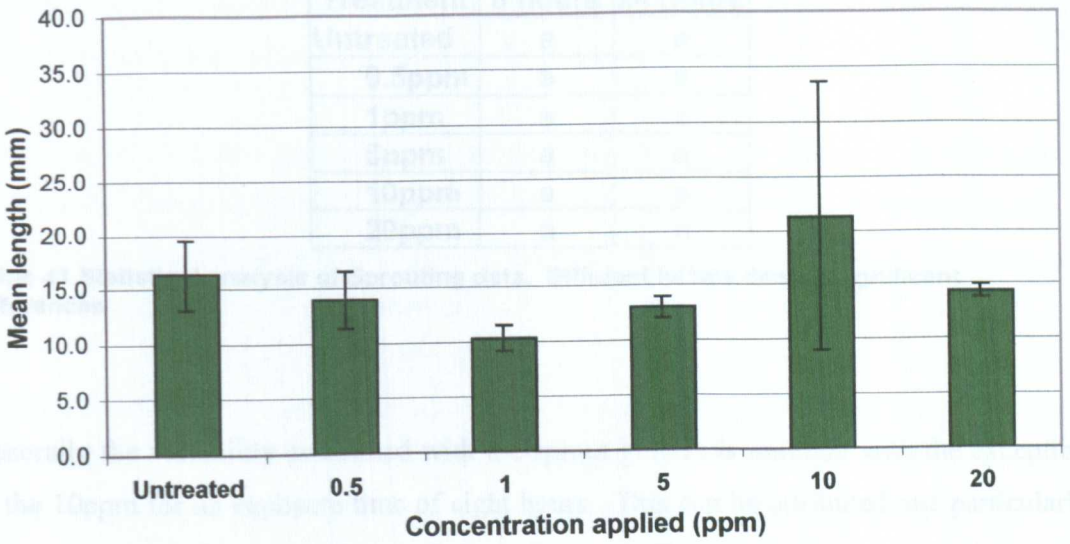


Figure 47 Mean length of the longest sprout-8hour exposure time (n=3)

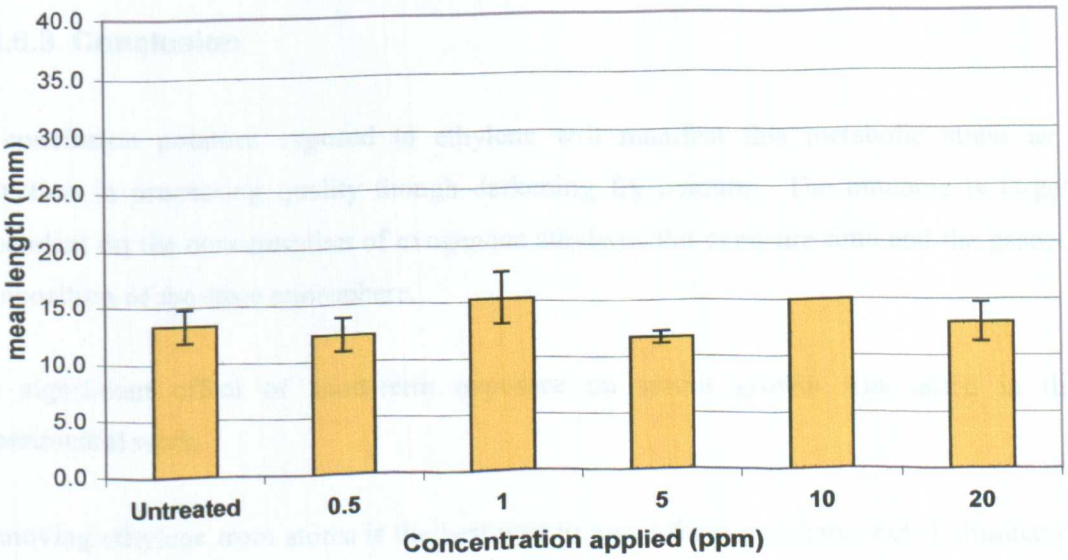


Figure 48 Mean length of the longest sprout 24hour exposure (n=3)

Treatment	8 hours	24 hours
Untreated	a	a
0.5ppm	a	a
1ppm	a	a
5ppm	a	a
10ppm	a	a
20ppm	a	a

Table 41 Statistical analysis of Sprouting data. Different letters denote significant differences

Generally the variability associated with the sprout growth is minimal with the exception of the 10ppm for an exposure time of eight hours. This can be attributed one particularly high mean value from a replicate of the treatment. This arose from a few tubers with extensive sprout growth within the set of 10. The high mean value cannot be removed as an outlier because the sample set is not large enough to permit this.

There was no obvious trend in sprout growth resulting from exposure to ethylene for 8 or 24 hours. There did not appear to be any distinguishable difference between the untreated and any of the ethylene treatments at either ventilation time.

5.1.6.3 Conclusion

In conclusion potatoes exposed to ethylene will manifest this metabolic stress as a reduction in processing quality though darkening fry colours. The outcome is largely dependant on the concentration of exogenous ethylene, the exposure time and the gaseous composition of the store atmosphere.

No significant effect of short-term exposure on sprout growth was noted in this experimental work.

Removing ethylene from stores is the best way to avoid these problems, but if elimination is not possible, dilution by ventilation can minimize its effects (*Saltveit, 1999*).

5.1.7 Conclusions

Potatoes produce ethylene naturally in very low levels. Exogenous sources of ethylene give rise to increased evolution of ethylene from tubers.

The findings of these experiments confirmed absolutely the presence of ethylene in commercial storage facilities at physiological active levels following thermal fogging. It became clear that the ethylene was present from the process of thermal fog generation and was not a direct result of CIPC treatment.

The impact of ethylene exposure on fry colour is negative and the extent of this darkening in appearance is heavily dependant on the duration of exposure. The concentration within the range expected from thermal fog application has less of a determining role. Further work is required to investigate the full effect of ethylene on potatoes in storage.

6 Chapter Six

6.1 CONTINUOUS EXPOSURE OF POTATOES TO AN ETHYLENE ATMOSPHERE

6.1.1 Introduction

The initiation of sprouting in potato tubers is accompanied by a variety of biochemical changes. These are usually reflected in fluctuations in hormonal concentrations, respiration rate and the onset of nucleic acid synthesis and cell division and enlargement. *Rylski et al, 1974*, found that both short-term and long-term (8, 24 hour & 40 days) exposure to ethylene gave rise to a substantial increase in respiration rate, which peaked in every case twenty-four hours after treatment started. Sprouting appeared only to be stimulated by eight-hour and twenty-four hour exposure. Long-term exposure completely retarded sprouting for the duration of treatment, but after ethylene application was discontinued sprouting ensued at an identical rate to that in the tubers which received brief exposure to the gas. However sprout morphology was different. Long-term exposure had inhibited elongation of sprouts. *Rylski et al* concluded that both short and long-term exposure shortens the duration of rest but only long-term exposure inhibits bud elongation. This is an example of the difference between the perception and the synthesis of ethylene, which can differentiate between the responses elicited.

The interpretation of arrested growth of the sprouts in the continual presence of ethylene, is essential in understanding that this apparent reduced sprouting is conditional upon the presence of a critical concentration of ethylene. *Abeles, 1973* highlights that this is not indicative of it being an effective technique for preventing sprouting in commercial storage as accelerated sprouting would occur on return to normal air when crop is sold.

A change in sprout morphology is common to tubers that have been previously exposed to ethylene for a considerable time (*Prange et el, 1998*).

Figure 49 Tubers from GU trials in which they were exposed to an external source of ethylene.

The photos above are from a GU trial in which there was little evidence of apical dominance. These smaller, more widespread rosette type sprouts resulted from removing tubers from continued ethylene exposure. Another common feature of tubers previously exposed to ethylene is lateral branching of sprouts.

Although progressive loss of apical dominance is a normal consequence of tuber ageing (*Coleman, 2000*), the loss of apical dominance illustrated is related to the auxin concentration (a plant hormone that is involved in the control of many aspects of plant behaviour including the suppression of lateral buds). Numerous processes are controlled by ethylene in a close interaction with auxin, and often it is impossible to differentiate between ethylene and auxin effects.

> "Ethylene production is frequently stimulated by auxins and the relationship between ethylene production and the level of either applied or endogenous auxin is often an extremely close one" *(Hill, 1980)*.

Although auxin is known to stimulate the production of ethylene at high concentrations, it is unclear whether it is a requirement for ethylene stimulation. It is possible that auxin and ethylene interaction could result from regulation of sensitivity and transport rather than synthesis. Thus, auxin production rate could have different effects on ethylene sensitivity in specific tissues and at particular stages of development (*Smalle & Van Der Straeten, 1997*).

An example of the different types of behaviour expressed in different situations is given by *Hill, 1980*. He states that ethylene production in geotropically stimulated pea stems is

related to the auxin content of these tissues, but is not directly responsible for the observed growth response. Whereas in root tissue geotropic stimulation increases ethylene production, which affects root growth almost instantaneously. This is a result of auxin redistribution due to geotropic stimulation leading to enhanced levels in certain parts of the root. Ethylene production increases because of the higher auxin levels in these tissues. The ethylene produced inhibits growth in the localised area, thereby altering growth of the root until ethylene levels return to normal. Thus the relationship in different tissues (stems and roots) of the same plant can be very different.

Smalle & Der Strenen, 1997 concluded that ethylene treatments have a profound impact on vegetative development. They deduced that endogenous ethylene plays a less critical role in comparison to other plant hormones such as auxin or gibberellins.

Earlier work (*Alam et al., 1994*) found that exogenous ethylene also released bud dormancy in potato, but from protein profiles suggested it was by an indirect means, by accelerating or enhancing the action of other hormones. Similarly:

> "It has an effect on the synthesis of some enzymes and nucleic acids but some of its effects on plants are so rapid that nucleic acid and protein synthesis are unlikely to be involved in the first instance" (*Hill, 1980*).

The potato tubers response to ethylene can be complex and dependent upon whether the response is mediated by the level of internal ethylene production (i.e. synthesis) or merely the direct result of exposure to exogenous ethylene. It is my opinion that this is the reason long-term exposure can retard sprouting, yet still cause an increase in reducing sugar concentration. This is exemplified by the onset of vigorous sprout growth on the removal of tubers from an ethylene atmosphere. Suggesting the effect on sprouting is by direct means unlike the reducing sugar accumulation, which is believed to be the result of increased biosynthesis. Often the findings in literature on this subject are conflicting and rarely can clear decisive patterns of behaviour be extrapolated from the conclusions drawn in individual papers. Nevertheless, ethylene is vital in the control of plant response to environmental stresses.

6.1.1.1 Exogenous Ethylene

Synthesis of ethylene is possible from a number of compounds, most commonly ethanol, in the presence of either;

sulphuric acid at high temperatures

$$CH_3CH_2OH + H_2SO_4 \rightarrow (CH_3CH_2OSO_2H) \rightarrow CH_2CH_2 + H_2SO_4$$

aluminium oxide at high temperatures

$$CH_3CH_2OH \rightarrow CH_2CH_2 + H_2O$$

Potentially considerable amounts of carbon monoxide can be formed when sulphuric acid is used, therefore the preferred method for generation is with a metal catalyst (*Abeles, 1973*).

These sources of ethylene as well as pressurised gas cylinders have been used in trials studying the effect of ethylene on tubers with particular interest in using it as a potential sprout suppressant chemical.

Generally burning any fuel will generate ethylene as a by-product (*Abeles, 1992*). Combustion gases created in the standard procedure for thermal fogs contain ethylene.

Prange et al, 1998 reported that reducing sugar concentration is increased and hence the darkening of fry colour occurred as a result of exogenous ethylene exposure.

Exogenous ethylene is known to have an autocatalytic effect on ethylene production. This stimulatory effect can be reduced by the presence of high concentrations of carbon dioxide (2-20%) (*Chevery et al, 1988; Mathooko, 1996, 7*).

Unfortunately carbon dioxide in potato stores in levels above 3% will induce a darkening of fry colour through increasing reducing sugar concentration (*Mazza and Siemens, 1990; Briddon and Jina, 1999).*

Research on the effect of exposure time by *Hughes, Timm and Weaver, 1986*, concluded that sprout vigour was reduced by comparison with the control treatment in potatoes exposed to ethylene at 1ppm for periods of longer than 3 days. Potatoes stored for up to

twenty-six days continued to sprout less when exposed to 1ppm ethylene than those not exposed to ethylene.

6.1.2 Continuous exposure experimental work

This was studied with the intention of showing whether the effects of continual exposure were different to short-term exposure as illustrated in literature (*Rylski et al, 1974, Suttle, 1996, Prange et al, 1998, Jeong, 2002*). The first investigations at GU of ethylene exposure on stored potatoes for this project were done using a simple continual source, apples. Apples are known to produce high amounts of ethylene. The general pattern reported was increased reducing sugars and altered sprout morphology as a consequence of ethylene exposure via presence of apples (*Shah, 1997*).

Another option to investigate continuous ethylene exposure was to use a slow release compound. A growth enhancing liquid available commercially for use on crops was selected. Firstly the slow releasing ethylene compound had to be tested. This was a substance called Ethrel C from Crop safe (480g/l 2-(chlorethyl) phosphonic acid). When the Ethrel is exposed to an alkaline environment it initiates release of ethylene gas. The rate of gas release is dependant upon the ratio of Ethrel solution to alkaline solution. Ethylene gas will be released until all of the starting material is consumed, after this point the Ethrel solution will have to be refreshed.

6.1.2.1 Preliminary experiments

6.1.2.2 Using apples as an ethylene source

In an undergraduate project supervised by me apples (Golden Delicious) were placed in storage boxes with the potatoes at the start of the trials and numerous treatment variables followed. The student that carried out the investigation *(Glennie, 2001)* was aiming to find out what the effect of continual exposure to a relatively low concentration of ethylene was on the behaviour of potato tubers.

Ethylene is an important ripening promoter in fruits and is evolved prior to the respiratory climacteric minimum (*Agatsuma and Tamura, 1973*). *Meigh et al, 1967*, compared apples that had been cut from trees to apples that remained attached and found that those that were cut had a lower respiration rate but produced more ethylene. The level of ethylene production was fairly constant. This shows that damage to fruit (abscission) results in increased ethylene production. The same effect is shown by *Abeles, 1973*, with rose flowers. Damage is a common means of generating increased ethylene levels in plant tissue.

Design-Part 1

Firstly the amount of ethylene released from the apples was studied within an oxygen restricted environment and an unrestricted environment using 2l chromatography tanks for this purpose. They had glass lids with septum ports inserted through them to facilitate headspace analysis.

Anoxic conditions were achieved by sealing the lids on top of the tanks using PTFE tape, overlaid with sellotape. In this sealed environment a more realistic idea of the amount of ethylene evolved by the apples was gained in the initial period until the oxygen supply becomes limited. The aerobic storage conditions were more similar to the conditions in a potato store and provided data on what the expected release rate of the apples will be in the storage trial with potatoes. To do this cardboard spacers were placed around the perimeter of the tanks and the lids placed on top of these with tape to hold the lids in place.

The tanks were stored at 9°C for twenty-eight days. The treatments were carried out in duplicate.

Method-Part 1

Each tank contained one fruit (Golden Delicious).

2ml headspace samples were withdrawn using a gastight syringe on days 0, 1, 8, 13, 17, 21 and 27 of storage. The end of the needle was blocked with a septum for the time taken to transport the sample to the laboratory for injection onto the GC column. In all cases this was under one minute. Loss from the syringe in this time was negligible (The GC analysis was done using the first machine described in the materials and methods section).

Results and discussion-Part 1

Ethylene production from the apples in the sealed environment was fairly constant for the first two weeks of storage generating an atmospheric concentration of 35ppm and 55ppm respectively. The two different levels of production were thought to be due to differences in individual fruits used. The rate of production slowed after approximately fifteen days, considered to be a result of depleting oxygen supplies limiting the ethylene production within the apples. However the atmospheric levels lingered in the region of 30 and 42ppm respectively for the remaining two weeks of storage.

Aerobic storage conditions resulted in headspace concentrations of less than 0.20ppm (therefore hard to quantify exactly) in the first few days, but after 8 days had accumulated to approximately 0.25ppm. The levels in both containers were relatively stable at this approximate concentration for two weeks, then displayed a small increase to nearer 0.35-0.40ppm until the end of the trial.

Apples have been shown to provide a consistent supply of ethylene for a considerable period, therefore making them suitable for use as a practical and continual source. Even when exchange with ambient air occurs the storage atmosphere maintains measurable levels of ethylene.

Design-Part 2

Approximately 6kg of potatoes (Cultivar Cara) were placed inside 18l cardboard boxes. Some of the boxes contained apples for a given period of time, as stated below in *Table 39*, which details the treatments investigated. Again the treatments were carried out in duplicate. The boxes were stored at 9°C for a twenty-eight day period.

Treatments
Apples only
Potatoes only (control)
Potatoes with apples for 7 days
Potatoes with apples for 21 days
Potatoes with apples for 28 days

Table 42 Summary of treatments involving ethylene exposure using apples as a source

As the volume of the boxes was much greater than that of the tanks used in part 1, but the exchange with ambient air (via leakage) is estimated to be similar the number of fruits used had to be increased to allow measurable levels of ethylene to be reached. In this case three fruits were used per box receiving ethylene treatment. Also a concentration greater than 1ppm was desired in the boxes as the prime observation was the effect on sprouting with a view to using ethylene to achieve sprout control. Concentrations lower than this would have been more likely to stimulate growth rather than retard it.

Methods-Part 2

The ethylene concentration in headspace was measured by taking 2ml samples with a gastight syringe. As before the sample needles were plugged with a septum until injected directly into the GC for analysis (within a minute of collection). These samples were collected on days 0, 1, 3, 7, 10, 14, 21 and 28 of storage.

Respiration was measured on one occasion. The carbon dioxide level in the boxes was determined at the end of the trial before the final ethylene samples were collected and the boxes were emptied.

Tuber samples were collected on days 1, 7, 14, 21 and 28 of storage for reducing sugar analysis following gas sampling of the headspace. Three potatoes were taken from each box per sampling occasion. The weight removed was recorded to ensure results expressed on the basis of fresh weight remaining in the box could be correct (respiration). The samples were assessed for reducing sugar content by the Roe, Leloir-Roe and Somogyi-Nelson methods described in the Methods chapter.

Sprouting observations were noted throughout the storage period and photographs taken to illustrate the findings at the end of the trial.

Results and discussion-Part 2

The first graph below (*Figure 50*) is the mean headspace ethylene concentration of the treatments investigated.

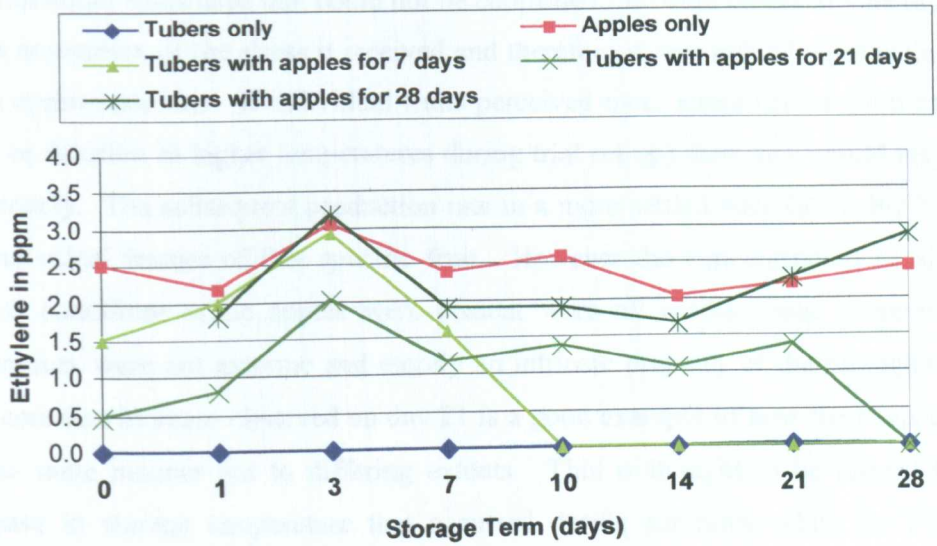


Figure 50 The mean atmospheric ethylene concentration in boxes resulting from treatment

It is clear from the graph that the tubers alone in the box did not generate any measurable level of ethylene (in these sampling conditions) for the entire duration of the trial. The apples alone produced the most consistent ethylene concentration in the box atmosphere and with the exception of two sampling occasions (day 3 and 28) had the highest concentration throughout.

All boxes containing apples had peaked in ethylene concentration by day 3 between 2.0-3.0ppm. After this time the rate of production is assumed to have slowed as the concentration lowered in all cases. This initial burst of ethylene synthesis was probably a result of the stress of handling the apples to set up the trial. From day 7 and beyond the concentration that had been reached in the box atmospheres was maintained (within +/–0.5ppm) until the apples were removed. The decline in concentration is obvious from the 7-day and 21-day treatments in the graph. After removal of the apples the levels in boxes dropped to below anything quantifiable.

In the boxes that had apples in for the full 28 days the concentration of ethylene was sustained until the end of the trial.

There was a different concentration of ethylene produced in separate boxes although the number and variety and source of fruit used was the same. This was a biological feature of the individual fruits used that could not be controlled. It could potentially be related to the fruits perception of the stress it received and therefore it responded by producing ethylene at an appropriate rate. If individual fruits perceived more stress (in the form of handling, time or duration in higher temperatures during trial set-up) then they would respond more vigorously. The subsequent production rate in a more settled state (after day 3) would be an individual feature of that specific fruit. However the concentrations in all boxes in settled conditions while apples were present were all in the range 1-3ppm. So any differences were not extreme and merely an intrinsic property of the biological produce. The common increase observed on day 21 is a good example of how the fruits all respond in the same manner but to differing extents. This is thought to be related to a slight increase in storage temperature that occurred during sampling when the localised air temperature was higher than on the other sampling occasions.

The graph uses the mean values, but no error bars are included for the very reasons discussed above. Firstly the concentration was quite individual to each box, depending on the fruits inside and the difference in exposure concentration (1-3ppm) was not large. Secondly the respiration and reducing sugar results are expressed per treatment, encompassing both replicates of each treatment, so to reflect this both replicates are averaged for presentation. A further major factor influencing this choice in presentation is that each of the treatments (excluding the control) produced the same approximate ethylene concentration and therefore exerted the same effect on the tubers while they were exposed. The variable being tested in this experiment is the length of exposure to ethylene.

The treatments performed as intended. The tubers in those boxes that did not contain apples were not exposed to ethylene at all. Whereas the tubers in the other boxes were exposed to ethylene until the apples were removed. The above points are general observations. No statistical analysis was performed on this data.

The reducing sugar results did not produce any comprehensible pattern. This is thought to be a consequence of problems associated with carrying out the method of analysis, rather than a true reflection of the reducing sugar concentrations in the tubers themselves.

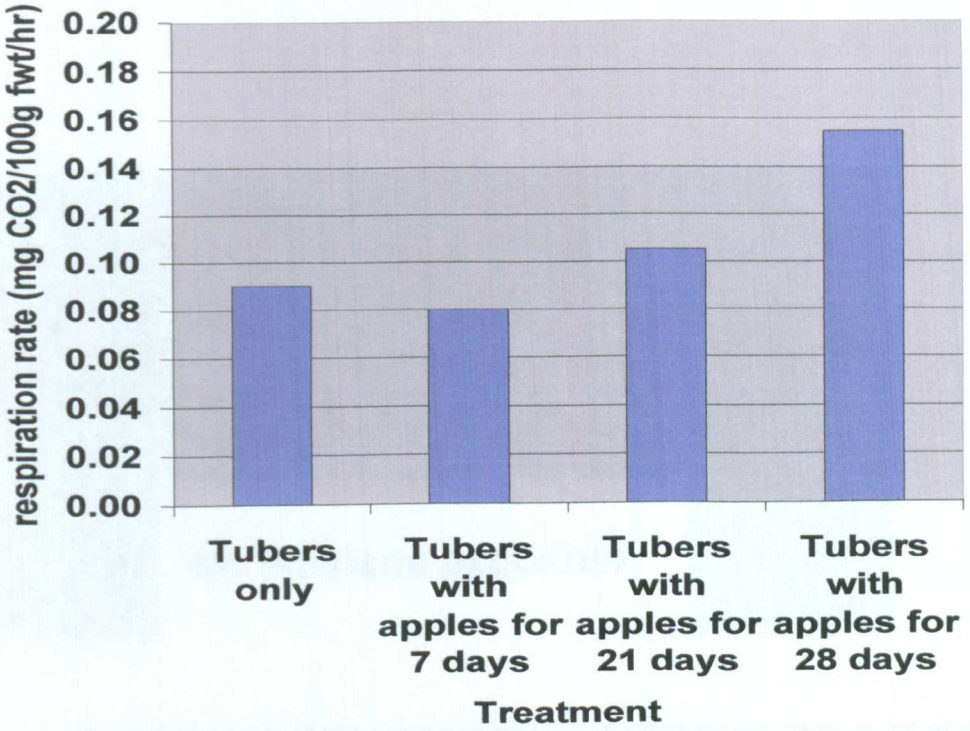


Figure 51 Mean respiration rates on day 28 resulting from treatments

Clearly the amount of respiration was much greater in the boxes that still contained both tubers and apples for the 28 days, as the apples were present during sample time. Comparing the data, with the exclusion of the sample exposed for 28 days, shows there was not a great difference in respirations rate of tubers between treatments, suggesting the presence of ethylene in these concentrations did not significantly alter respiration.

The sprouting observations were the most interesting results to come from the trial. They plainly illustrated in the following sequence of photographs that when tubers were exposed to ethylene under these conditions that sprout growth could be inhibited. The retardation of sprouts was dependant upon the continual presence of ethylene in this narrow concentration range. Shortly after the ethylene source was removed sprouting ensued at an equal rate to those tubers in non-ethylene treated environments. Also evident is the influence that an ethylene atmosphere had on the morphology of the sprouts and the existence or not of apical dominance.

Figure 52 Sprouting on tubers that were not exposed to ethylene for the duration of the trial

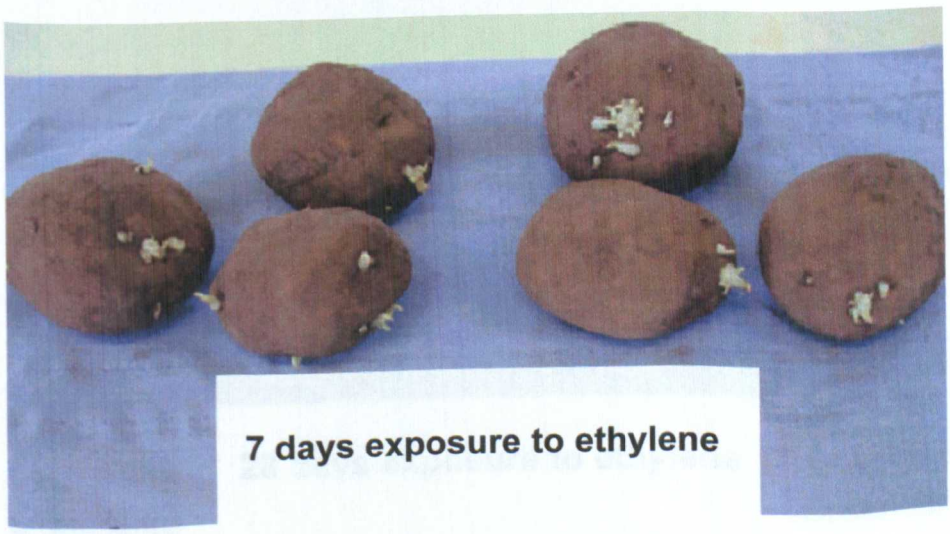


Figure 53 Sprouting on tubers that were exposed to ethylene for the first seven days of the storage term

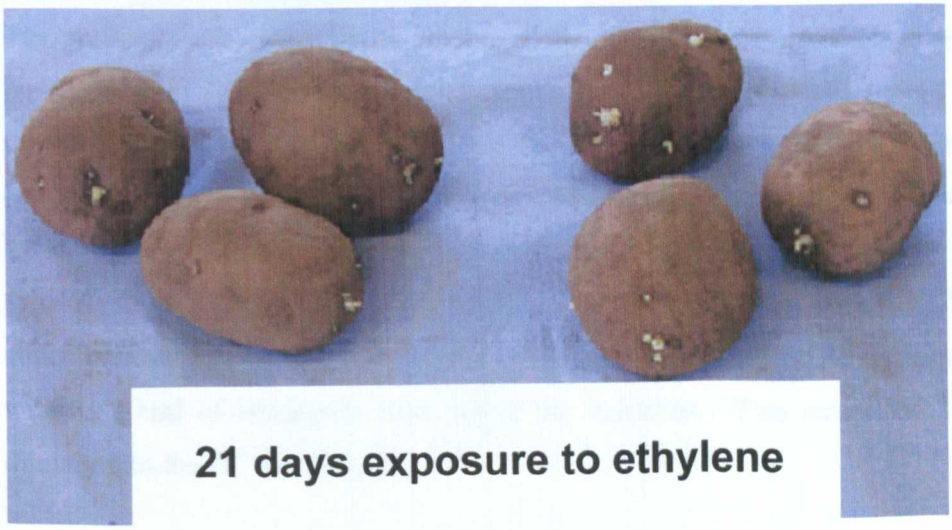


Figure 54 Sprouting on tubers that were exposed to ethylene for the first 21 days of the storage term

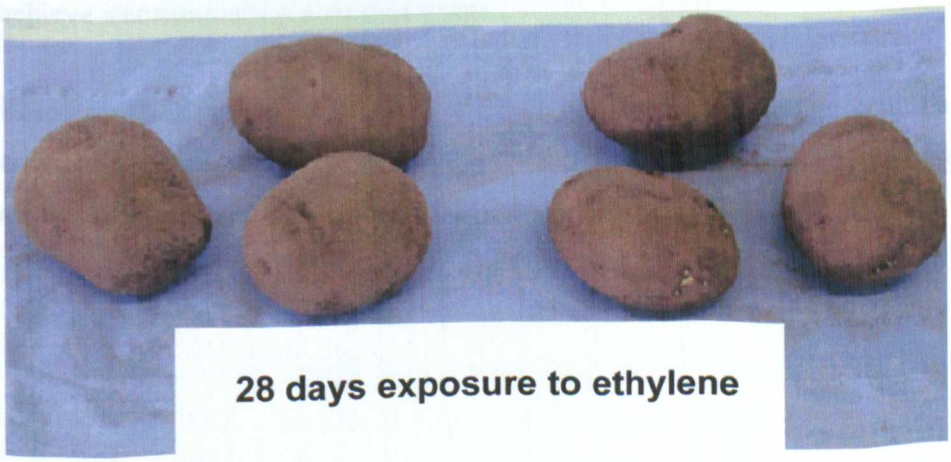


Figure 55 Sprouting on tubers that were exposed to ethylene for the entire duration of the storage term (28 days)

Following on from this student's work it was decided to be to the benefit of this project if investigations into continued exposure to ethylene were repeated in more controlled conditions.

6.1.2.3 Ethrel C as slow release form of ethylene

The preliminary experiments to determine the rate of release of ethylene from the Ethrel under different conditions were conducted in headspace bottles. The yield of ethylene at different pH's was tested first (by an undergraduate student working under my supervision). 50ml of 1:100, Ethrel:buffer solution was placed inside each headspace bottle. The buffer solutions used were pH 4, pH 7, pH 9.2 and pH 11. For each pH value three replicates were set up. The headspace bottles were stored at 10°C. The headspace concentration was tested after 24 and 48 hours. A 2ml gas-tight syringe was used to withdraw 1ml of headspace from above the solutions. This sample was then injected directly into the GC for analysis.

The results from this trial clearly indicated that:

Ethrel released more ethylene at higher pH levels

A good supply of Ethrel starting material needs to be present to provide a desirable atmospheric concentration of ethylene. The above 1:100 ratio was rather too little to achieve a concentration even near 1ppm.

Based on the conclusions of the student's work all the subsequent solutions required for continued release of ethylene were made using 10mls of Ethrel and adding a relatively small predetermined volume of saturated sodium carbonate (Na_2CO_3).

Investigation of the control of ethylene release from Ethrel C

The influence that the volume of alkali releasing agent had on the rate of gas production was established before any storage trials were carried out. The following volumes of saturated sodium carbonate were added to the 10ml of Ethrel: 0.5, 1.0, 5.0, 10.0, 20.0 and 40.0ml. Each treatment was duplicated in 120ml headspace bottles. The volume of headspace was calculated for each volume added and used to determine the concentration of the ethylene.

ratio	headspace volume (ml)
10:0.5	109.5
10:1.0	109.0
10:5.0	105.0
10:10.0	100.0
10:20.0	90.0
10:40.0	70.0

Table 43 Headspace volume remaining in the bottles after both solutions are added

The volume of headspace gas extracted with the gas-tight syringe had to be altered depending on the concentration inside the bottle. So for the lower volume additions of alkaline 2ml samples were collected and for the larger additions a lesser volume was removed for sampling (1ml, 500µl or 10µl). These gas samples were injected directly onto the top of the GC column.

The results are included in the following tables. The first table (*Table 46*) shows the overall mean concentration in the headspace of each ratio tested. In the second table the amount of ethylene produced has been calculated as a release rate over the time since the previous sample was collected. In some cases there are values missing from the tables these values are not included as they were not sufficiently reliable due to problems with GC separation on one of the duplicate samples. Even with missing values the general pattern is quite clear. The more alkali added the more ethylene was released within a short time scale. There was less difference between ratios at the upper end of the range tested.

Mean concentration in the headspace of each set of bottles (µl/l)				
	10:0.5	10:1.0	10:5.0	10:10.0
24 hour	0.98	4.59	21200.72	22419.20
48 hour	79.45	22.39	21235.10	
7 day	3.49	12.34	24612.57	42556.15

Table 44 Ethylene concentration in headspace generated by different Ethrel to saturated sodium carbonate ratios

The concentration in the headspace for the highest of the ratios tested was ~23020 and ~25560µl/l after 24 hours for 10:20 and 10:40 respectively. There were no further values for these two variables.

release rate µl/l			
ratio	24 hr	48 hr	7 days
10:0.5	0.0045	0.3580	-
10:1.0	0.0208	0.0808	-
10:5.0	92.7531	0.1504	0.2833
10:10.0	93.4133		13.9840
10:20.0	86.3251		
10:40.0	74.5500		

Table 45 Release rates of ethylene from the different solution ratios over time

It seems there is an optimum range for the volume of addition of saturated sodium carbonate that will yield the maximum release rate. This optimum range is between 5 and 10ml added to 10ml of Ethrel for immediate release. Over a longer period the best ratio was 1:1 or 10ml Ethrel to 10ml saturated sodium carbonate. This ratio provided the highest rate of release over the 7 days. In each case the rate of release had slowed substantially between 2 and 7 days. In two cases (lower end of ratios tested) the ethylene was not being produced any longer or was being depleted faster than it could be produced, this circumstance is indicated by a minus sign. The blank spaces are the times when the results were not reliable or reproducible. In these cases the rate of release cannot be commented upon as not enough detail is known.

6.1.2.4 Using Ethrel C as an ethylene source

It was decided that greater control of the concentration of ethylene in the storage atmospheres could be gained using Ethrel as a source rather than apples. Therefore it was used for the main trial studying the effects of long-term exposure to ethylene on the quality of potatoes in storage.

Design

The potatoes (Cultivar Saturna) were held in storage at 10°C for a 28-day period. The concentration of ethylene applied was different for each treatment. The levels chosen were relative to each other and labelled high, medium and low. A control was included which involved no exposure to ethylene.

Each treatment was replicated three times. Therefore a total of twelve 18l storage boxes were used. 3kg of potatoes was placed inside each box. The boxes were placed in the relevant incubators and allowed to settle for three days in the storage conditions before the trial began. Samples were collected for reducing sugar analysis on days 0, 1, 7, 14 and 28 of the trial period. When the boxes were opened for tuber collection the sprouting observations were carried out. These included recording the percentage of tubers that were sprouting, the length and morphology of sprouts and any apparent orientation of sprouts relative to the ethylene source.

Method

The actual ethylene concentration was monitored throughout the trial. Headspace gas samples were taken twice weekly from each box and analysed by GC. The volume collected from each box was dependant upon the concentration aimed for inside the box. So for the high concentration boxes a small volume was withdrawn whereas for the low concentration a larger sample volume was used (up to a maximum of 2ml). This was done to prevent having samples over the calibrated range of the GC for ethylene determination. A gastight syringe of appropriate volume was used for collecting headspace samples.

The Ethrel plus releasing agent solutions were prepared immediately before placing inside the storage boxes and returning them to the incubators. The mixing of solutions generated quite an effervescent reaction. The more alkali added the more vigorous the reaction. For this reason it was performed under an aluminium foil lid placed loosely on top of the

beaker with a hole pierced in it to allow the pipette tip through to deliver the releasing agent. This procedure was done as quickly as possible to ensure that most of the initial burst of ethylene was not lost before the solution was placed inside the boxes. Solutions were refreshed every three or four days systematically for the duration of the trial.

The three different concentrations of ethylene in the box atmospheres (low, medium and high) were achieved by adding different volumes of saturated sodium carbonate to the Ethrel. Each time the mixed solutions were prepared 5ml of Ethrel was first placed into the beaker (~30ml capacity). To this the predetermined volume of releasing agent was added via pipette. The volumes used are detailed in *Table 48*. They are expressed as a ratio of Ethrel to saturated sodium carbonate, both in (ml).

Treatment	Low	Medium	High
Ratio of volumes	5:0.05	5:0.5	5:2.5

Table 46 The ratio of Ethrel to releasing agent for a range of ethylene concentrations (set 1)

As the headspace concentration of ethylene on days 0, 1 and 5 was below the limit of quantification for all treatments the ratio of releasing agent to Ethrel solution was adjusted to allow more ethylene to be produced. The mixed solutions prepared from 5 onwards used the volumes detailed in the table following (*Table 49).*

Treatment	Low	Medium	High
Ratio of volumes	5:2.5	5:5.0	5:10.0

Table 47 The ratio of Ethrel to releasing agent for a range of ethylene concentrations (set 2)

The reason the ethylene produced from the first set of solutions was not quantifiable could have been that:

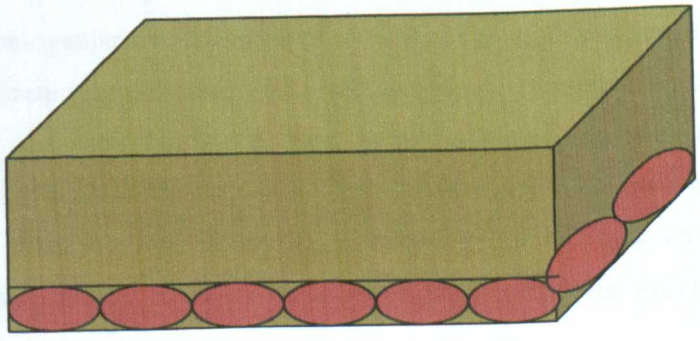
There was not sufficient alkali solution to release an adequate supply of ethylene.

The substantial air movement within the growth cabinet was flushing away the ethylene more rapidly than it could accumulate.

Increasing the volume of alkali releasing agent used allowed ethylene to be released more rapidly and overcame both of these factors.

The box volume was 18l. It is assumed that only $^1/_4$ of the box volume had tubers in it i.e. the bottom 4.5l contained the tubers. The air space between the tubers was estimated to be roughly 55% of this volume. At the start of the trial there was 3kg in each box in one layer. On each sampling occasion (days 1, 7, 14 and 28) approximately 10% of the starting weight was removed (~300g), therefore the airspace between tubers was increased by approximately 10% of its original value following sampling occasions. This is not entirely accurate as once tubers have been removed the remaining tubers occupied a full layer on the base of the box. However it does provide a good approximation for calculating the total air space within the box at each stage of headspace sampling. This information together with the atmospheric concentration (determined from headspace samples) was used to work out the total ethylene in the box air

The green area represents the total air space. This includes the free air above the potatoes and the air between the tubers.



The tubers occupy approximately one quarter of the space within the box.

At the start of the trial:

Total volume:18l

Tubers + air between tubers:4.5l

Free air:13.5l

Air between tubers = 55%:2.5l

Total air volume: 16l

Space occupied by tubers = 45%:2.0l

On each sampling occasion ~10% of tubers are removed =0.2l of space

Therefore on the sampling days stated below the total air volume is as calculated:

Day 7: 15.8l

Day 9 and 15: 15.6l

Day 18 and 27: 15.4l

For analysis of reducing sugar content two tubers per box were removed, washed, peeled and diced. A representative ~25 g subsample was taken from the mixed cubes of the two tubers. This sub sample was homogenised with methanol to extract the soluble sugars and filtered under vacuum. The filtrate was collected and made to volume in methanol. An aliquot was then analysed by the Roe, Leloir-Roe and Somogyi-Nelson methods for reducing sugar content. The full details of methods used can be found in the Materials and Methods chapter. From these three sets of analyses the common reducing sugar levels used as an indication of processing quality in the potato industry (glucose and fructose) can be calculated.

Results and Discussion

The mean values from the headspace results are displayed in the tables below. There are no results for the control (no ethylene) as when sampled there was no detectable ethylene present in the storage atmosphere on any sampling occasion.

Table 50 indicates the total amount of ethylene present in the store air. *Figure 56* represents that value expressed as an atmospheric concentration. From *Figure56* it becomes obvious that the treatments labelled low and medium were not different in terms of the concentration of ethylene generated by the conditions of the trial. Concentrations in both the low and medium treatments fluctuated and at times the low treatment actually created more of an ethylene atmosphere than the medium. This is thought to be for two reasons. Firstly, the volume of releasing agent added did not permit the release of significantly different amounts of ethylene from the Ethrel solution. Secondly the air movement within the outer storage facility may have influenced the residence time of ethylene inside the boxes. The boxes were stacked in three layers, a single column of three boxes for each treatment. All nine of the ethylene treated boxes were stored inside one growth cabinet with substantial air movement. When the solutions were refreshed the positioning of the columns was altered to ensure no one treatment was subject to more air movement than the others.

The high treatment consistently generated a higher concentration of ethylene in the boxes headspace than the other two treatments.

Mean volume of ethylene evolved over time from each treatment (µl)					
Treatment	Day 7	Day 9	Day 15	Day 18	Day 27
Low	30.39	19.45	8.94	31.06	17.66
Medium	26.33	12.17	19.03	15.55	9.24
High	106.60	36.04	64.22	68.94	136.70

Table 48 The total volume of ethylene present in the headspace of boxes

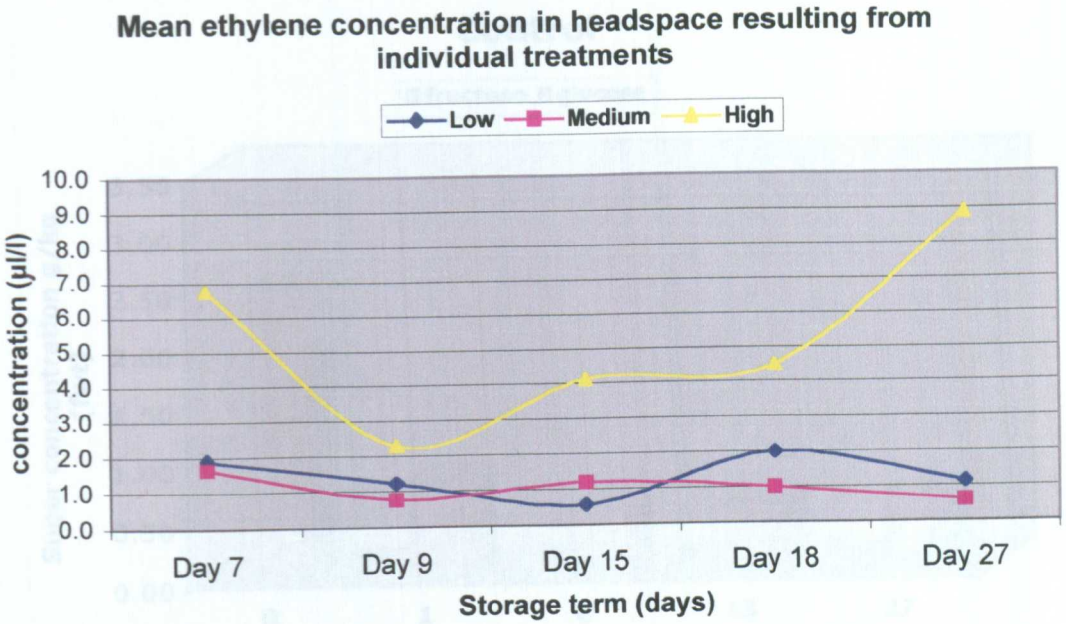


Figure 56 The mean ethylene concentration in headspace of boxes resulting from individual treatments

Both low and medium treatments did allow the potatoes to be continually subjected to ethylene in the range 0.5ppm to 2.0ppm. Whereas the concentration created by the high treatment fluctuated to a greater extent between 2.0ppm at the lowest up to 9.0ppm at the highest. The times when the solutions were refreshed can be seen clearly from the high treatment line on the graph (Day 0, 5, 9, 14, 18 and 23). Headspace samples were collected prior to refreshing of solutions.

Basically all treatments except the control fulfilled the requirements of continual ethylene exposure from day 7 of storage onwards.

Tubers from only two of the treatments had sprouted by the end of the 28-day trial. The mean percent of remaining tubers that had sprouted was 12.3% at approximately 1.2mm length from the control treatment and 3.1% at approximately 0.8mm under the high ethylene treatment.

The following reducing sugar results are presented as the mean reducing sugar (fructose plus glucose) concentration of the replicates from each treatment over time.

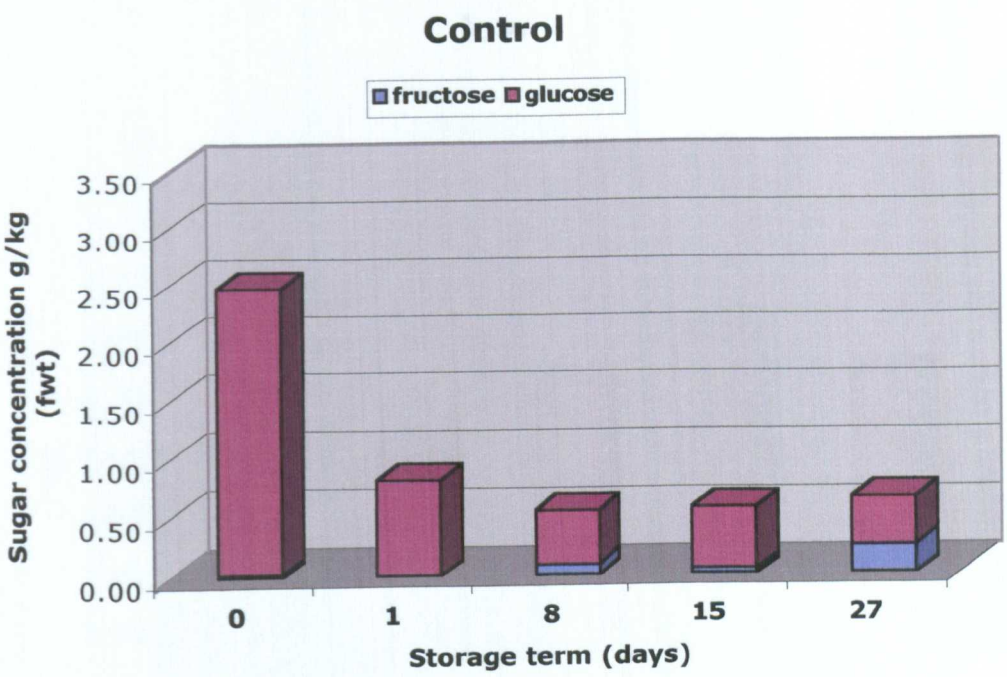


Figure 57 Mean reducing sugar concentration resulting from storage conditions

The high level of reducing sugars noted on day 0 is the result of handling stress, and is more or less consistent across all treatments. This is followed by a steady decline toward a generally stable level of approximately 0.5g/kg, which is low by comparison to the other treatments.

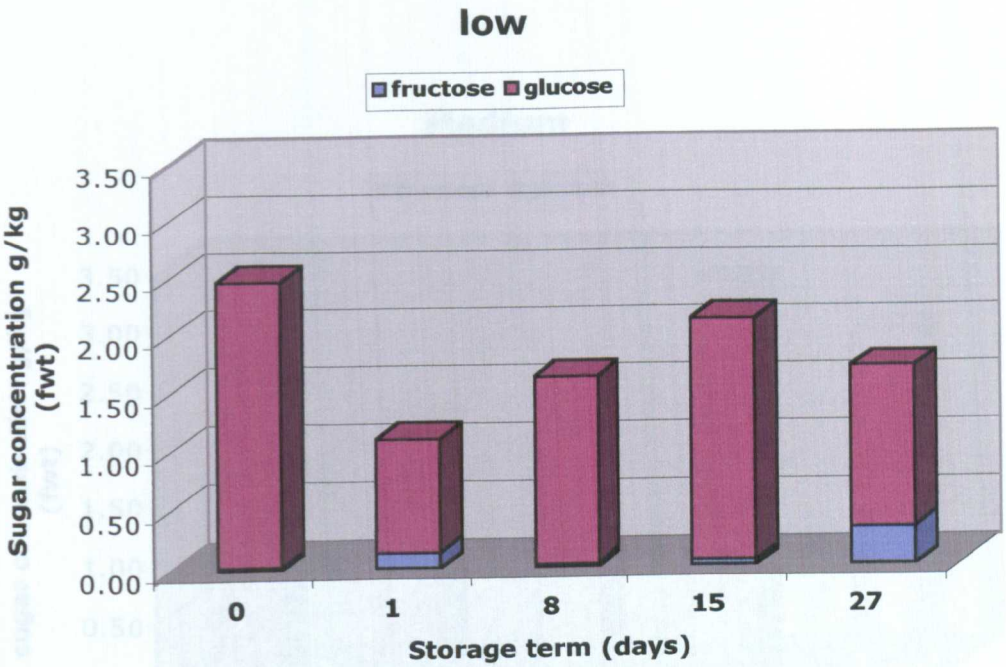


Figure 58 Mean reducing sugar concentration of samples exposed to ethylene in storage (low)

After the initial handling response there was a rise in reducing sugar levels due to ethylene exposure. The ethylene concentration levels do not exactly follow the sugar levels at the same time and potentially a lag time between exposure and manifestation of an effect on sugar levels exists.

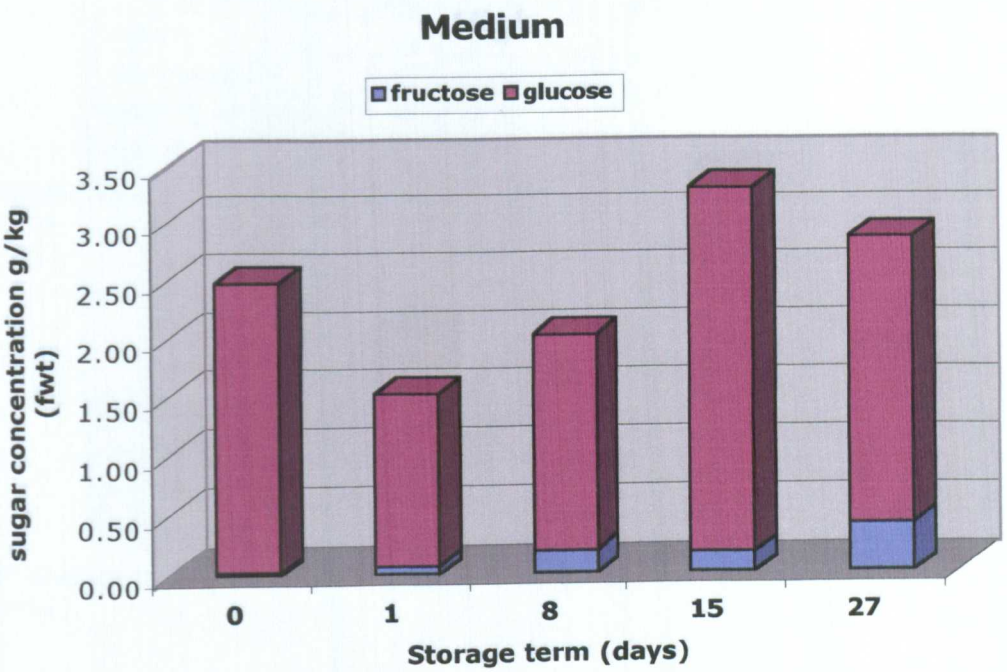


Figure 59 Mean reducing sugar concentration of samples exposed to ethylene in storage (medium)

The pattern is similar to the previous graph but with consistently higher levels. Although for days 7 and 9 the associated ethylene levels are very similar in both treatments. Where the largest increase in reducing sugars is noted on day 15 the ethylene concentration is twice that in the former graph. Regardless of concentration the pattern of sugars is the same. A clear increase is evident by comparison with the control treatment. This treatment is significantly different to that of the control on days 15 and 27.

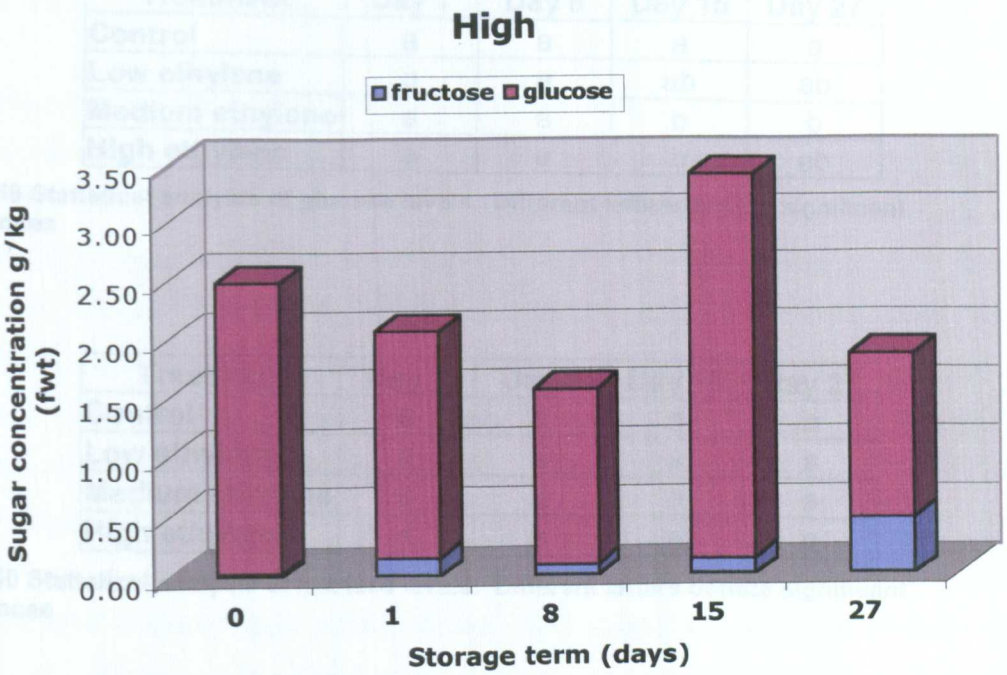


Figure 60 Mean reducing sugar concentration of samples exposed to ethylene in storage (High)

The ethylene concentration in this treatment was higher and the peak reducing sugar concentration was reached as a result of this. Only on the day the concentration peaked (day 15) was this treatment significantly different to the control. There was no significant difference in the overall sugar levels between all three of the ethylene treatments.

The potential lag between peak ethylene and the highest sugar level was more pronounced here with ethylene at 6.8ppm on day 7 and the reducing sugars peaking at 3.3g/kg on day 18.

Above a certain value the concentration of ethylene may not have any greater impact on reducing sugars or fry colour.

Each time the glucose levels were much higher relative to fructose and so they appear to respond more to ethylene treatment. This could be indicating starch degradation.

Treatment	Day 1	Day 8	Day 15	Day 27
Control	a	a	a	a
Low ethylene	a	a	ab	ab
Medium ethylene	a	a	b	b
High ethylene	a	a	b	ab

Table 49 Statistical analysis of glucose levels. Different letters denote significant differences

Treatment	Day 1	Day 8	Day 15	Day 27
Control	a	a	a	a
Low ethylene	a	a	a	a
Medium ethylene	a	a	a	a
High ethylene	a	a	a	a

Table 50 Statistical analysis of fructose levels. Different letters denote significant differences

6.1.3 Commercial store using ethylene as a sprout suppressant

6.1.3.1 Introduction

A well-known commercial potato storage company in the UK has recently been developing ethylene as a means of suppressing sprouts in relatively low temperature stores. The idea behind this work is that 'residue free' potatoes can be provided to supermarkets that increasingly demand less or no pesticides be used. These large supermarkets expect potatoes of superior quality (disease free, taste, texture, turgidity and aesthetically) to be provided throughout the year without the use of pesticides. They will discriminate not only on a quality basis but also on the use of pesticides. The augmenting pressure on growers and store managers has caused a renewed interest in alternatives to CIPC for suppressing sprouts.

Using ethylene is perceived as a 'residue free' method because the ethylene is present in the store atmosphere as a gas and at no point will a particulate be deposited on the surface of the tubers. Therefore analysis of residual ethylene in the tuber would be very difficult (although it most certainly penetrates the skin of the tubers in the gaseous form). Adding to this is the fact that ethylene is a natural plant hormone and is present (and synthesised) within most plant tissues in small amounts already. So differentiating between normal hormonal ethylene and applied exogenous ethylene would be virtually impossible. Any method would have to be unobtrusive, as stressing the tubers would cause the natural production to increase and would most likely skew the results. Thus, not only is ethylene a natural compound and as such more acceptable in the public eye, but the low gaseous concentrations in air will disperse readily and leave no truly quantifiable residue on the potatoes. The registration of ethylene for this purpose will have to be made through the Pesticide Safety Directorate (PSD), but the category under which it is registered may be open to some debate owing to its hormonal existence.

The downside of using ethylene as a sprout suppressant is the fact that it will lead to an increase in reducing sugar concentration in potatoes and ultimately render darker fry colours. This negative effect on processing quality is substantial and means this application is unacceptable for crop intended for processing markets. However it is expected that further research into the use of ethylene blocker compounds, to limit the detrimental impact on sugars, could lead to expanding applications for ethylene as a sprout suppressant.

An additional use for ethylene in the potato industry that is growing in popularity is the treatment of seed potatoes to maximise their potential for growth. This angle along with the sprout suppression attributes of ethylene highlights its behaviour as a hormonal growth regulator.

6.1.3.2 Design and Method

The ethylene atmosphere in a 200 tonne store was achieved using an ethylene generator similar to those used in the fruit industry. The starting material was denatured alcohol, which was dropped onto a hot metal catalyst at a predetermined rate to create an atmospheric concentration of approximately 10ppm. No extra controlled atmosphere conditions were required, only a sustained concentration of 10ppm (+/– 2ppm) of ethylene in air. Stores had to be relatively leak-proof to prevent the ethylene from diffusing freely to outside air, but cannot be completely sealed or a potentially dangerous build up of pressure would occur. The store was held at a crop temperature of approximately 3-4°C.

The concentration in the store was monitored using gas sensors (DBInstruments) that intrinsically are not as sensitive as GC analysis. When the concentration was found to reach trigger levels (down to 8ppm or up to 13ppm) the generator would be stopped or started as required helping to maintain the desired 10ppm. The data from the gas sensors was sent to a computer (DBInstruments) that plotted the mean ethylene concentration over time. It was apparent from the graphs produced that the greatest fluctuations occurred overnight, but overall the concentration was consistently within the range of 8-13ppm. The company wanted to know more precisely the concentration of ethylene in the store atmosphere. They requested analysis of a store atmosphere sample by GC to determine this.

A single opened 5l sample tin was placed inside a store for a few hours before sealing with the lid and removing from the store. The tin was packaged and sent to GU for analysis by GC.

Upon arrival (within 48 hours) the tin was placed in an incubator at 4°C for 90 minutes while the GC was calibrated. 2ml gas samples were withdrawn from the tin using a gas-tight syringe. Three replicate injections were made.

6.1.3.3 Results and discussion

The results are in the table below with the mean and associated standard deviation.

Ethylene concentration in tin (ppm)	
mean	**9.30**
standard deviation	*0.150*

Table 51 Ethylene concentration in tin sample of commercial store atmosphere

The mean concentration of ethylene in the tin was deemed to be representative of the store atmosphere. It was within the expected range and the reproducibility of the injections was good. These results show the method to be reliable for sampling store atmospheres in these conditions. The company for whom the sample was analysed were satisfied with the results, suggesting it correlated well with their own data from gas sensors, although this was never confirmed.

6.1.4 Conclusions

The continuous exposure of potatoes to ethylene in the approximate concentration range of 2-5ppm was successful in suppressing sprouting for the duration of exposure. When the potatoes were returned to normal air sprouting commenced fairly rapidly.

Both ethylene sources tested in this chapter of experimental work behaved in an identical manner as far as the tuber responses were concerned. Obviously there was more control of concentration and delivery rate using the slow release solution. In work presented in the previous chapter it was shown that ethylene supplied from a standard pressurised gas cylinder of specific concentration was also sufficient for this purpose. The suggestion being that any source that generates and maintains an adequate uniform atmospheric concentration would be suitable for work of this type, experimental or commercial. In most cases uniformity of concentration is not a major limitation because ethylene mixes very well with air, but this will depend to an extent on temperature.

There does not seem to be any additional benefit or drawback with using concentrations greater then 5ppm, but this would presumably vary with cultivar and environmental conditions. Using concentrations way in excess of those expected to be present in thermal fog may well bring about tuber breakdown and should be avoided. Relatively low concentrations of <1ppm are more likely to have a sprout promoting effect than suppressing. In this context, it has been reported that ethylene can be used for breaking tuber dormancy, by combining selected concentrations and exposure times (*Vacha & Harvey, Coleman & M'Inerney, 1997, Coleman, 1998).*

Commercially, ethylene storage would not be suitable for processing crop. The use of an ethylene blocker or antagonist compound would possibly allow this application in the processing sector.

Having thoroughly examined the range of effects that ethylene can have on potatoes in long-term storage and what factors can influence these effects, the problem of how to ameliorate the decline in quality following hot fog treatment were considered at a practical level.

7 Chapter Seven

7.1 POTENTIAL METHODS CONSIDERED FOR REDUCING THE IMPACT OF THERMAL FOG APPLICATION ON PROCESSING QUALITY

7.1.1 Introduction

Having established the negative impact that standard thermal fog applications of CIPC have on the processing quality of potatoes in storage, methods of ameliorating the problem were studied.

The aim was to reduce or prevent the increase in reducing sugar concentration commonly observed after fog treatments are applied. The earlier work on the fogger system (*Chapter 4*) demonstrated that it is the use of combustion products to create the fog (generated from burning petrol) and more specifically ethylene introduced from this process that leads to an increase in reducing sugar concentration in the tubers. Many concepts that would theoretically lessen the ethylene interference were considered, however for practical and economic reasons the simplest approaches were attempted first.

These included:

Ventilating sooner following applications thereby not allowing the combustion products to be in contact with the potatoes for as long a period. The idea being, if the exposure time was reduced the amount of stress on tubers would also be reduced and hence a smaller increase in sugars would be observed.

Generating less contaminants like ethylene by using a purer fuel source that is considered more environmentally friendly.

Altering the amount and frequency of combustion gases going into stores by tailoring the dose of CIPC applied per treatment.

Removing the harmful contaminants from the store atmosphere before they have the opportunity to exert stress on the potatoes.

In collaboration with staff at SBEU and Stored Crop Conservation, who kindly donated the cost of all applications, these trials were conducted successfully. In some cases the work was repeated at a later date in the year to confirm the findings and show how the physiological status of the crop affected the outcome.

More complicated and costly methods of reducing the impact of fogging that were not studied as part of this project but may lead to improvements include:

Adding catalytic converters to the fogger to completely oxidise the small hydrocarbon compounds (like ethylene) and reduce any potentially harmful nitrate compounds. This would have to be fitted inside the fogger after the combustion gases pass through the flame trap and before the CIPC formulation is delivered into the stream of hot gases. In theory this should remove the majority of contaminants from the fog before it enters the store. The effectiveness of this would have to tested and most likely a number of additional factors (e.g. distance from the combustion chamber, flow-rate, contact time) would need to be examined to find the optimum operating conditions.

Using a source of power to heat the air that does not involve combustion products. An electric heat exchanger can do this, but requires a tremendous amount of energy and consequently costs are high. The efficiency of such systems is low unless under strictly controlled conditions. Several CIPC applicators in UK are researching and developing heat exchanger machines with the aim of 'emission free' applications. The process is expensive and a substantial power source has to be available for any normal commercial sized application. In many stores power sockets cannot be easily accessed and a generator would have to be brought on site by the applicator. The logistics become more complicated as the equipment required increases in size and weight.

In the United States and in some parts of continental Europe using systems of this type are already used in common practice, where larger store sizes help to justify the cost of larger more demanding equipment. In the long-term the UK market will most likely move toward this type of application, but it may be some time before an acceptable machine is ready for commercial use.

Pre-treating crop with compounds that antagonise/inhibit the ethylene biosynthesis or response could in theory minimize the increase in reducing sugar concentration following exposure. These compounds are used predominantly to study the biosynthesis and activity of ethylene, but can be adapted for commercial value on food and ornamental crops. A

range of compounds can be used (e.g. aminoethoxyvinylglycine, aminooxy-acetic acid, 2,5-norbornaniene, silver ions, diazocyclopentadiene and a number of cyclopropenes). They differ greatly in effectiveness based on how specifically they bind to the ethylene receptors and the time they remain bound which is controlled by the diffusion rate (*Sisler & Serek, 1999*). The rate of production of new receptors must be considered.

Control of store conditions at SBEU was done using the integrated Cornerstone System. This controlled relative humidity and temperature to within 0.3°C of the set-point.

7.1.2 Investigation of methods of control

7.1.2.1 Experiment 1: Ventilation Times

The objective was to determine the effect of timing of ventilation post-application on processing quality. Components such as ethylene can remain in store air for up to twenty-four hours. Therefore by ventilating stores earlier than the standard twenty-four hours after application the exposure time can be reduced.

Method

Experimental work was first conducted in May 2001 at SBEU using cv. Saturna stored at 10°C and 95% relative humidity. CIPC (50% w/v in methanol) was applied using a Unifog at a rate of 0.5l per store (12 tonne capacity) and 0.18l per 4.38 tonne storage container. Samples were transferred to an untreated store 8 or 24 hours after treatment, simulating ventilation. In May all replication of treatments were performed in 12 tonne stores. The untreated is used as the control.

This experiment was repeated in November 2001 under identical conditions, except that the control was treated with a CIPC dust formulation instead of remaining untreated. This dust treatment was necessary because the new crop had not previously received any sprout suppressant application. The CIPC dust (1% active ingredient) was used at a dose rate of 2g/kg. Each treatment was applied to crop in a 12 tonne store and the duplicate treatment to crop in a 4.38 tonne storage container (small well sealed shipping containers).

The baseline quality of the tubers was higher in November because the crop was fresh. The storage time of the trial was extended from 28 days to 42 days to allow any recovery in fry colour to be followed through.

All chemical treatments were carried out in duplicate. In May three and in November four replicate samples were collected from each store per sampling occasion. The samples consisted of 10kg trays containing approximately 30 tubers. The fry colour of the samples, including untreated material was assessed 0, 1, 7, 14, 28, 35 and 42 days after application.

After processing assessments of samples were made for a) weight of crisps with an unacceptable fry colour and b) the colour (Hunter Lab) of samples was determined (after

removal of defects). Samples were fried, defects evaluated and colour assessed as per standard BPC/SBEU operating procedure.

The Hunter L value assigned to each samples is a measure of the brightness and is determined instrumentally based on the reflection of light. The higher the L value the lighter the crisp colour.

The percent fry defects are the fraction of the crisp sample removed because 50% or more of the surface area of an individual crisp is below acceptable colour (namely L-value 49).

Headspace concentrations of carbon dioxide were measured using a landfill gas meter with a limit of detection of 0.1% CO_2. Headspace ethylene concentrations were determined with the use of a Gastec hand held pump and selective colorimetric detector tubes (semi-quantitative method). Assessments were made prior to ventilation of stores following treatment application.

This method of assessment of ethylene is acceptable for the purpose of this trial, albeit only semi-quantitative. Originally samples were to be collected on a transportable medium and forwarded to GU for analysis by the fully quantitative method of Gas-Chromatography (GC). Maintaining sample integrity between collection and analysis proved problematic. Therefore the Gastec method, which evades these problems, was undertaken. Good correlation was determined between GC and Gastec ethylene analysis during an earlier experiment carried out at GU. GC is the standard method of analysis for ethylene determination at GU and is proven to be reliable, reproducible and particularly sensitive.

7.1.2.2 Results and Discussion

The fry colour results presented are the mean values of each treatment encompassing all samples. Error bars display the mean value plus and minus one standard deviation.

May 2001

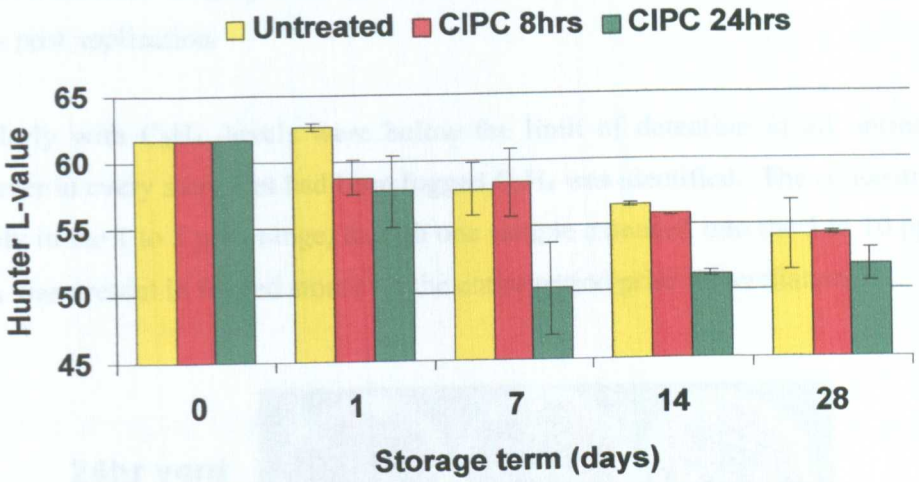


Figure 61 The effect of ventilation time on fry colour (n=4, May 2001)

Treatment	Day 1	Day 7	Day 14	Day 28
Untreated	a	a	a	a
CIPC 8 hour vent	b	a	a	a
CIPC 24 hour vent	b	b	b	a

Table 52 Statistical analysis of L values –May 01. Different letters denote significant differences

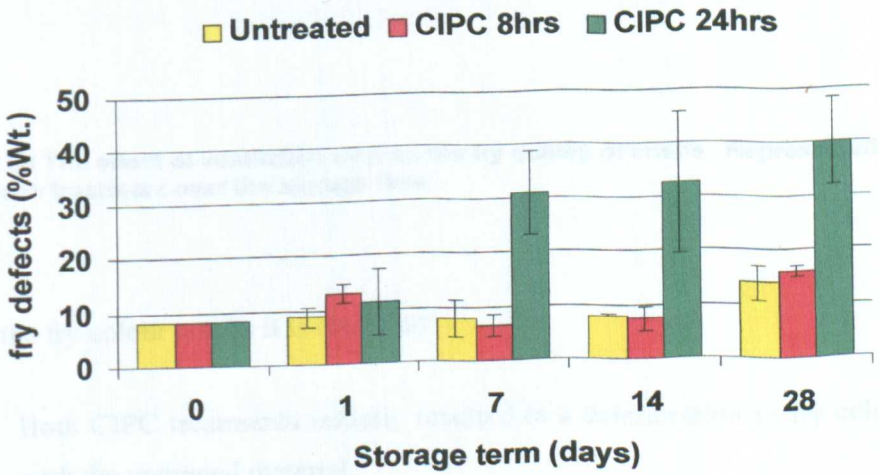


Figure 62 The effect of ventilation time on fry defects (n=4, May 2001)

The level of CO_2 in the untreated stores atmospheres was below the limit of detection for the duration of the sampling time. In all stores that received fog treatment CO_2 was found in concentrations ranging from 0.3 or 0.4% at eight hours to 0.4 or 0.5% at twenty-four hours post application.

Similarly with C_2H_4, levels were below the limit of detection in all untreated stores, however in every store that had been fogged C_2H_4 was identified. The concentrations were largely in the 1 to 5 ppm range, though one sample extended into the 5 to 10 ppm bracket. C_2H_4 was present in fogged stores for the entire period prior to ventilation.

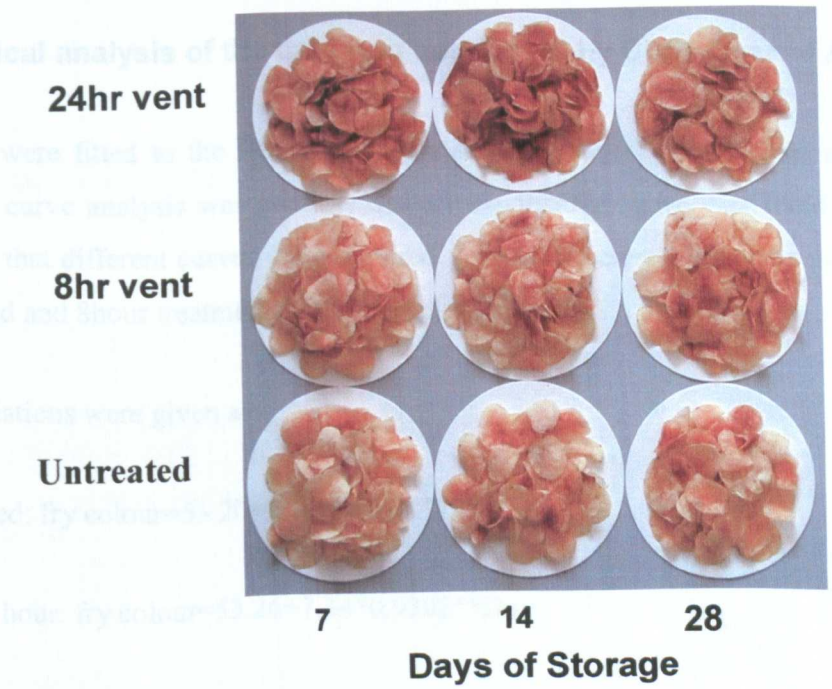


Figure 63 The effect of ventilation time on the fry quality of crisps. Representative samples from each treatment over the storage time.

From the fry colour results it is clear that

- Both CIPC treatments initially resulted in a deterioration in fry colour compared with the untreated material.
- During the period 7-28 days after treatment there was little difference in fry colour of untreated samples and those transferred 8 hours after fogging.

- Fry colour of samples transferred after 24 hours was significantly darker than the untreated between day 1 and day 14 inclusively.

- On day 1 the percent fry defects of all the treatments were significantly different.

- On all sampling occasions the percent fry defects of the samples transferred 24 hours after treatment were significantly greater than those of the untreated samples.

Fry colour and weight of defects of samples transferred after 24 hours remained poor for the duration of the study.

Statistical analysis of the data was conducted by Chris Dyer of ADAS

Curves were fitted to the Hunter L-values of each treatment over time and in this case parallel curve analysis was possible and an exponential curve was fitted. This analysis showed that different curves were required for each treatment, however the curves for the untreated and 8hour treatments were very close together.

The equations were given as:

Untreated: fry colour=53.20+9.12*0.9225**Day

CIPC 8 hour: fry colour=53.26+7.34*0.9302**Day

CIPC 24 hour: fry colour=51.06+10.72*0.587**Day

These accounted for 84.4% of variance in the results.

He advised that because of the low replication it was difficult to show significance on specific dates between treatments.

The nature of this research is such that replication within a trial particularly in limited storage space at SBEU and even more so in commercial facilities is virtually impossible to implement.

November 2001

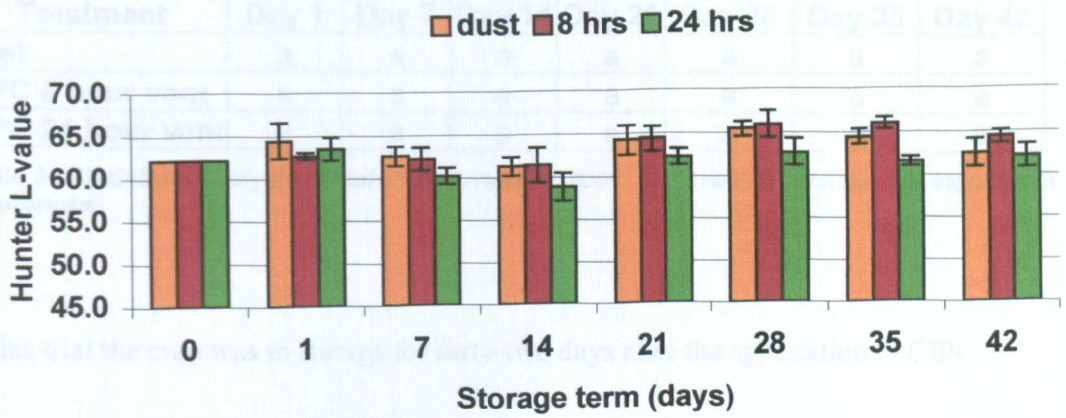


Figure 64 The effect of ventilation time on fry colour (n=8, November 2001)

Treatment	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Dust	a	a	a	a	a	a	a
CIPC 8 hour vent	a	a	a	a	a	b	b
CIPC 24 hour vent	a	b	b	b	b	c	a

Table 53 Statistical analysis of L-values-November 2001. Different letters denote significant differences

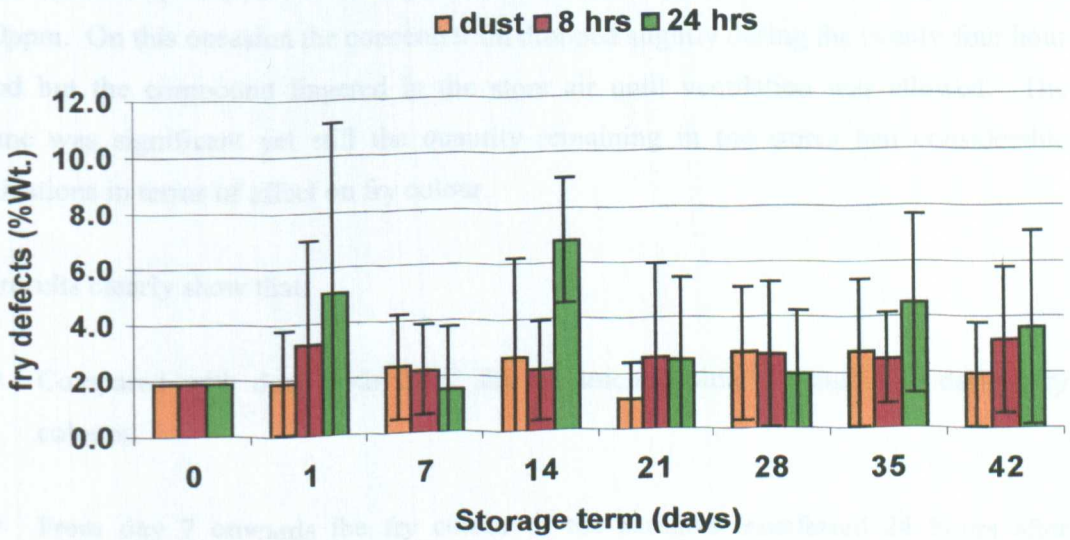


Figure 65 The effect of ventilation time of fry defects (n=8, November 2001)

Treatment	Day 1	Day 7	Day 14	Day 21	Day 28	Day 35	Day 42
Dust	a	a	a	a	a	a	a
CIPC 8 hour vent	a	a	a	a	a	a	a
CIPC 24 hour vent	a	a	b	a	a	a	a

Table 54 Statistical analysis of defects-November 2001. Different letters denote significant differences

In this trial the crop was in storage for forty-two days after the application of CIPC.

All treatments involved the application of CIPC. The standard treatment used as a control for comparison with fog treatments and ventilation times was CIPC dust. The use of a sprout suppressant chemical was essential for all crop to ensure a fair comparison of crop quality.

CO_2 levels were relatively low, but could be detected in each fogged store following application. The common, and maximum, concentration reached was 0.2% at eight hours post application. The levels remained at between 0.1 and 0.2% for the full twenty-four hours or until ventilation.

Again C_2H_4 was present in stores as a result of thermal-fog application, ranging between 1 to 10ppm. On this occasion the concentration dropped slightly during the twenty-four hour period but the compound lingered in the store air until ventilation was allowed. The decline was significant yet still the quantity remaining in the stores had considerable implications in terms of effect on fry colour.

The results clearly show that:

- Compared with dust, both CIPC fog treatments initially resulted in darker fry colours.
- From day 7 onwards the fry colour of the samples transferred 24 hours after treatment was significantly differently to the dust and the 8 hours samples
- On day 35 and 42 samples transferred 8 hours after treatment were significantly lighter than the dust treated samples.

- Overall the weight of defects was relatively low, but commonly samples transferred after 24 hours had the largest percent of fry defects.

- On day 14 the percentage defects of the samples transferred 24 hours after treatment were significantly greater than that of the 8 hour samples

Statistical analysis of the data was conducted by Chris Dyer of ADAS

Parallel line analysis indicated that separate lines are required for each treatment. The lines are:

Dust: fry colour=63.01+0.0243*Days

CIPC 8 hours: fry colour=62.07+0.0884*Days

CIPC 24 hours: fry colour=61.64+0.0051*Days

The fit for these lines was rather poor and only 28.6% of the variability was accounted for. Subsequently no significant differences can be stated with any confidence without further analysis.

7.1.2.3 Conclusions

Unfortunately the statistical package used for parallel analysis of results (Genstat) is not very easily transferred into a word document and summary findings had to be given.

The transfer of samples to an untreated store (simulating ventilation) 8 hours after conventional thermal-fog application of CIPC resulted in an improvement in processing quality of cv. Saturna at 10°C, compared with samples transferred after 24 hours.

Reducing the exposure time of crops in store to contaminants present in the fog successfully reduced the detrimental impact of thermal-fog application on processing quality. The extent of the improvement in fry colour depended on the age of the crop i.e. May samples were more sensitive to treatment effects compared to November samples. A similar point was raised in earlier research but it was discussing the ethylene production of tubers in relation to their age. *Okazawa, 1974* found that senile tubers released somewhat higher amounts of ethylene than the younger tubers. This was considered to be a result of their state of relative dehydration. It may be the case that the extra sensitivity later in the season is a consequence of elevated ethylene biosynthesis.

Current CIPC formulation label recommendations state that stores should be left sealed for a twenty-four hour period after application of a CIPC thermal-fog (with the exception of 2 labels, which allow ventilation after a 12 hour period). Therefore in this experimental work the eight-hour ventilation was not in accordance with the recommended procedure. However this eight-hour period allowed adequate time for the effective fraction of the thermal fog to settle.

The period of time required for the fog to settle had been previously determined for the stores in which the trials were conducted (*BPC Technology transfer leaflet*). The time required in individual stores would have to be determined, but is unlikely to be as long as twenty-four hours after application. Factors that must be considered are health and safety, environmental fate and efficacy. The label carries the recommendations for use of the product.

Data from this project was used to aid the case for updating CIPC label recommendations. Although that attempt was unsuccessful with one particular chemical company, the general understanding, resulting from technology transfer, among store managers and applicators is

that stores should be ventilated when the fog has cleared. In all cases this will be less than twenty-four hours.

Early ventilation is a simple and effective method that can limit the damage that common practice CIPC treatment has on fry colour and subsequently market acceptability.

7.1.2.4 Experiment 2: Alternative Fuels

The objective was to determine the effect of using an alternative fuel source, to generate a CIPC thermal-fog, on processing quality. Other aspects that must also be considered are fuel consumption, cost and practicality.

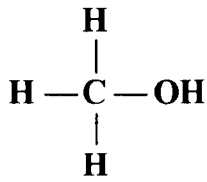
Burning cleaner, simpler hydrocarbon fuels should produce a less polluted flow of combustion gases. The simpler the fuel's chemical structure (smaller carbon skeleton) the less carbon dioxide and water vapour is generated per unit volume. By-products of combustion generally increase from gaseous to liquid to solid fossil fuels (*Ozdogan et al, 1997).* Essentially, smaller ranges of contaminants in lower concentrations are produced from purer fuels.

Pollutant emissions vary dependent upon running conditions, and in particular fuel/air ratio (*Abdel-Rahman, 1998*). A general increase in efficiency decrease the magnitude of pollutant emissions.

On this basis methanol and liquefied petroleum gas (LPG) were investigated as potential options for reducing the impact that fogging has on fry colour. Both methanol and LPG are considered to be the most important alternative fuels suitable for engine use (*Abdel-Rahman, 1998*). Currently the common fuel used to generate thermal-fogs in the UK is lead replacement petrol.

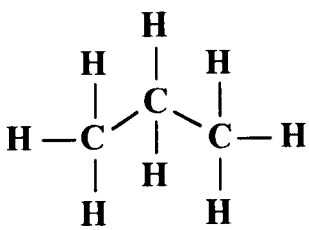
Methanol has the smallest molecular structure of the three fuels. It is considered a bio-fuel as it is a renewable energy resource. It was available for experimental use promptly as the adaptations required to the existing fogging machines were straightforward.

Structure of methanol



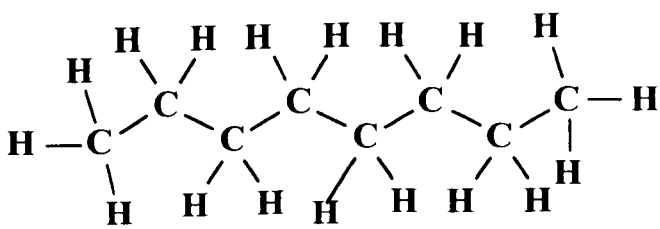
LPG is readily available and is at present used for many commercial purposes instead of petrol because it is both cheaper and cleaner to run. The more complicated process of adapting equipment to burn LPG meant that it was not ready for trials in May 2001, and after experimental trials in November 2001 still requires further work to optimize the system. LPG is already used for thermal fogging in the United States of America.

Structure of propane



The structure shown below is the nearest pure hydrocarbon structure to petrol. In reality, petrol is a very mixed and variable fraction of the distillation process and is a well-known source of pollutants.

Structure of octane to represent petrol



Method

All practical trials were carried out at SBEU using cv. Saturna stored at 10°C and 95% relative humidity. CIPC (50% w/v in methanol) was applied using a Unifog at a rate of

0.5l per store (12 tonne capacity) and 0.18l per 4.38 tonne storage container. Samples were transferred to an untreated store 24 hours after treatment, emulating ventilation.

The experiment was first conducted in May 2001. Untreated crop was used as the control and compared with crop treated using a petrol-fuelled fog (the standard fuel) and crop treated using a methanol-fuelled fog. All other conditions of application were identical. In May 2001 all treatments were conducted in 12 tonne stores.

Based on the findings from the trial in May and further developments in suitable fuel types the experiment was repeated in November 2001 with modifications to treatments. The treatments compared for the effect on processing quality were a petrol-fuelled fog (used in this case as the control), a methanol-fuelled fog and a LPG-fuelled fog. Where possible all other conditions of the application process were identical. Each treatment was applied to crop in a 12 tonne store and the duplicate treatment to crop in a 4.38 tonne storage container.

The baseline quality of the tubers (cv. Saturna) was higher in the second trial because the crop was fresh as it was the start of the storage season.

In May and in November three and four replicate samples respectively were collected from each store per sampling occasion. Each sample was a 10kg tray containing approximately 30 tubers. The fry colour of samples, including untreated material was assessed 0, 1, 7, 14 and 28 days after application.

All assessments were as in Experiment 1 encompassing fry colour, fry defects and headspace concentrations of carbon dioxide and ethylene prior to the ventilation of stores following treatment application.

7.1.2.5 Results and Discussion

The fry colour results presented are the mean values of each treatment including all samples. Error bars display the mean value plus and minus one standard deviation.

May 2001

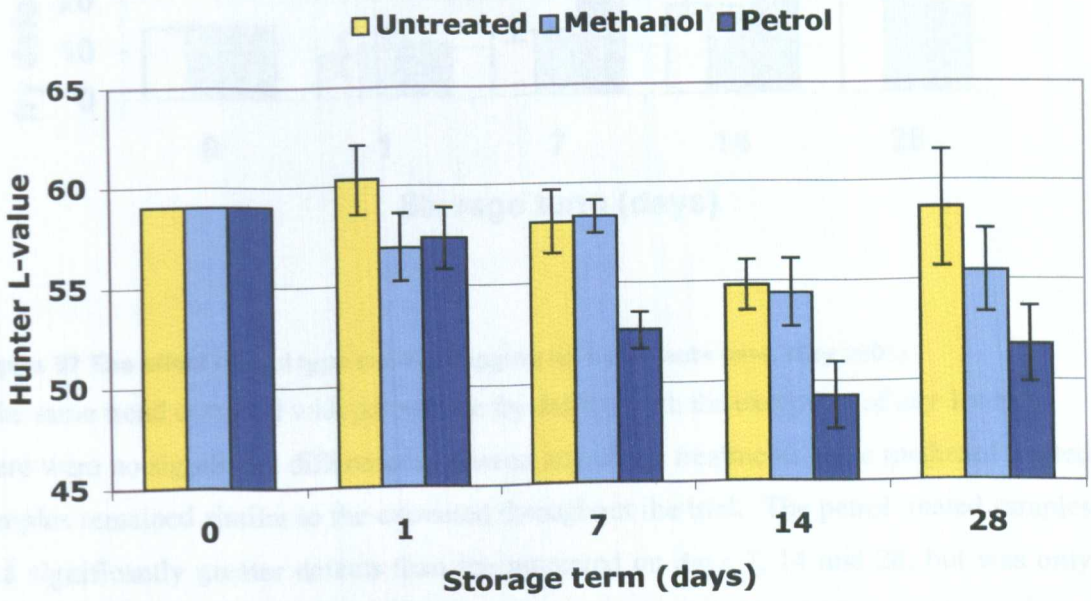


Figure 66 The effect of fuel type used in fogging on fry colour (n=4, May 2001)

Treatment	Day 1	Day 7	Day 14	Day 28
Untreated	a	a	a	a
Methanol fuel	b	a	a	ab
Petrol fuel	b	b	b	b

Table 55 Statistical analysis of L-values-May 01. Different letters denote significant differences

Both fog treatments resulted in significantly darker fry colours to the untreated on day 1. The petrol fuelled fog caused L-values to be significantly different to the untreated samples for the entire duration of the trial. While after day 1 the methanol treatment generated fry colours that were statistically the same as the untreated. By day 28 the petrol treated samples were no longer significantly darker than the methanol treated samples.

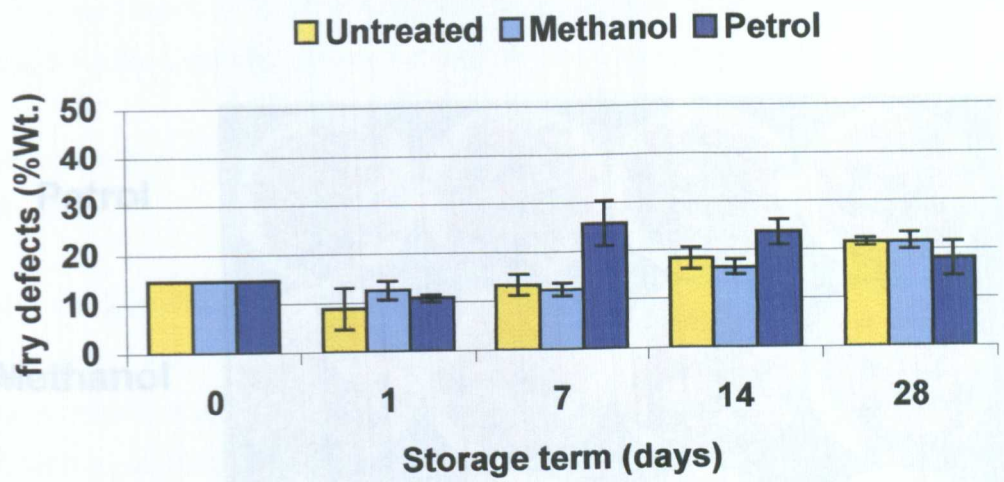


Figure 67 The effect of fuel type used in fogging on fry defects (n=4, May 2001)

The same trend occurred with percentage fry defects with the exception of day 1 when there were no significant differences between any of the treatments. The methanol treated samples remained similar to the untreated throughout the trial. The petrol treated samples had significantly greater defects than the untreated on days 7, 14 and 28, but was only significantly different to the methanol treated samples on days 7 and 14.

CO_2 concentrations in stores treated with petrol fuelled-fog were between 0.1 and 0.2% at both the sampling times (eight and twenty-four hours post-application). In those stores treated with methanol fuelled-fog CO_2 was between 0.1 and 0.3% on both sampling occasions.

C_2H_4 was detected in stores treated with petrol fuelled-fog for the full twenty-four hour period prior to ventilation, in the range 1 to 5 ppm. Methanol fuelled-fog caused diffuse unquantifiable readings. The methanol propellant interfered with the colorimetric reagents.

CIPC applied using conventional petrol and methanol propellants initially resulted in a deterioration in fry colour. The processing quality of the petrol-fogged samples was clearly suffering as a result of petrol combustion. Generally the weight of fry defects was increased using petrol propellant.

The effects of all treatments appear to lessen slightly toward day twenty-eight. This is significant as the holding time for crop after CIPC application before it can be used for

human consumption is between twenty-one and twenty-eight days depending on the product label.

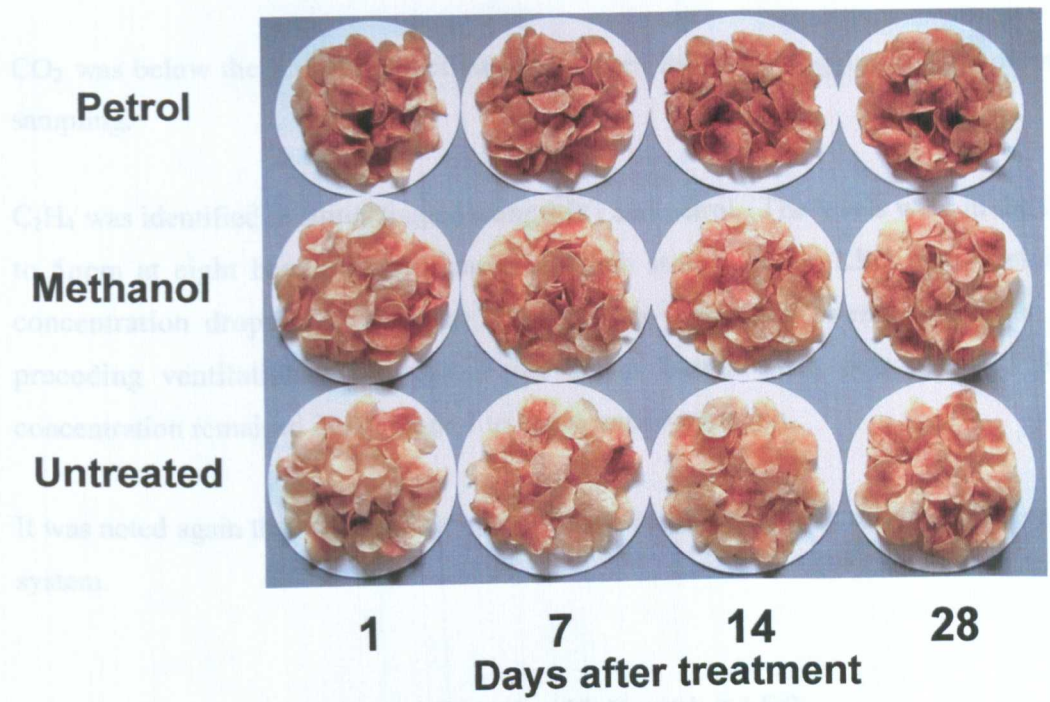


Figure 68 The effect of alternative fuel use on the aesthetic quality of crisps. Representative samples from each treatment over the storage term (May 2001)

Statistical analysis of the data was conducted by Chris Dyer of ADAS

Linear plus exponential curves were fitted to each treatment. These identify the minimum that occurs in all three treatments on day 14 and the increase for day 28. The equations are:

Untreated: fry colour=59.97+0.0123*1.265**Days-0.365*Days

Petrol: fry colour=58.35+0.0186*1.265**Days-0.711*Days

Methanol: fry colour=58.51+0.0056*1.265**Days-0.253*Days

They account for 81.0% of the variance

Again the small number of replicates has made it impossible to identify significant differences.

November 2001

In this experiment the CIPC treatments compared for the effect on fry colour were petrol fuelled-fog (the control), methanol-fuelled fog and LPG fuelled-fog.

CO_2 was below the limit of detection in all stores following fogging for the duration of sampling.

C_2H_4 was identified in stores fogged using LPG and petrol. The levels were in the range 1 to 5ppm at eight hour post-application in each store. In the LPG treated stores the concentration dropped to between 0.2 to 1ppm within the twenty-four hour period preceding ventilation. Whereas in the stores treated with petrol-fuelled fog the concentration remained in the range 1 to 5ppm until ventilation.

It was noted again that using methanol as caused interferences with the Gastec colorimetric system.

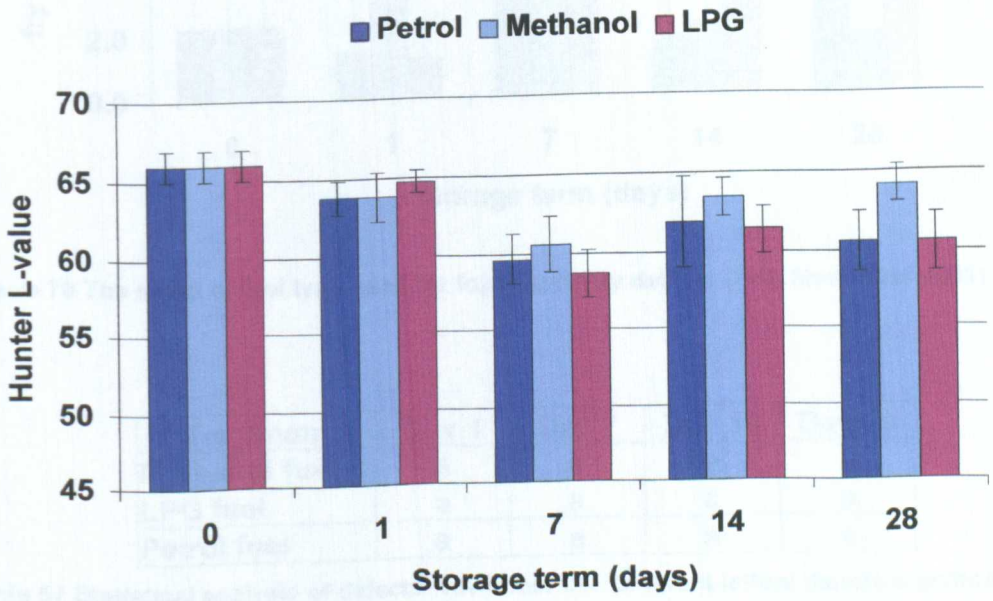


Figure 69 The effect of fuel type used in fogging on fry colour (n=8, November 2001)

Treatment	Day 1	Day 7	Day 14	Day 28
Methanol fuel	a	a	a	a
LPG fuell	a	a	a	b
Petrol fuel	a	a	a	b

Table 56 Statistical analysis of L-values-Nov 01. Different letters denote significant differences

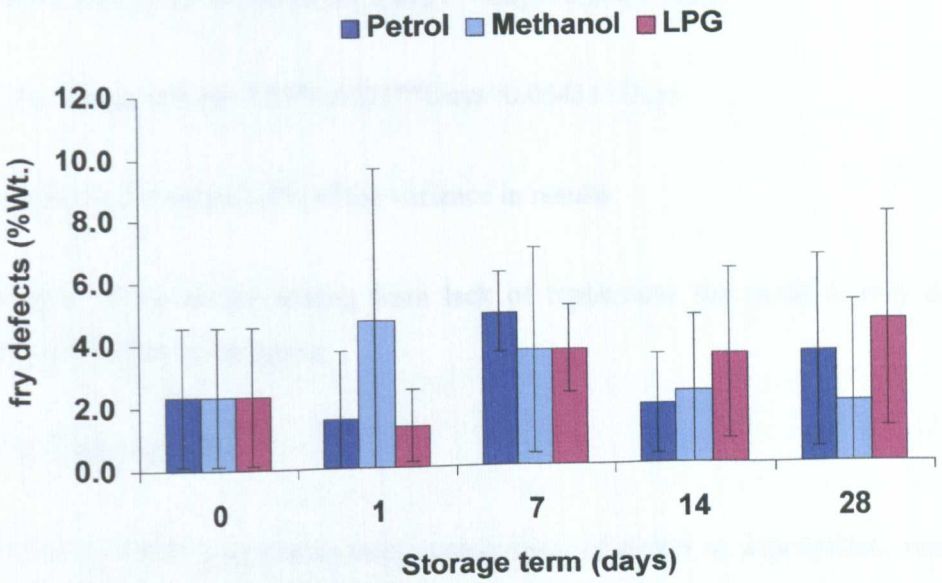


Figure 70 The effect of fuel type used for fogging on fry defects (n=8, November 2001)

Treatment	Day 1	Day 7	Day 14	Day 28
Methanol fuel	a	a	a	a
LPG fuel	a	a	a	a
Petrol fuel	a	a	a	a

Table 57 Statistical analysis of defects-November 01. Different letters denote significant differences

CIPC applied using conventional petrol, methanol and LPG propellants initially resulted in a deterioration in fry colour.

Methanol fuel was much less detrimental to processing quality than LPG or petrol, but was only significantly different on day 28.

The difference in effect on fry colour between CIPC applied using LPG and petrol was only slight.

Statistical analysis of the data was conducted by Chris Dyer of ADAS

Linear plus exponential curves were fitted to each treatment. The equations of the lines are:

Petrol: fry colour=60.21+5.82*0.6151**Days+0.02685*Days

Methanol: fry colour=60.00+5.96*0.6151**Days+0.1644*Days

LPG: fry colour=59.36+7.21*0.6151**Days+0.05411*Days

This accounts for only 61.9% of the variance in results.

The extent of variability arising from lack of replication has made it very difficult to distinguish between treatments.

7.1.2.6 Conclusions

Conventional CIPC application using combustion of petrol as a propellant resulted in a significant deterioration in fry colour and an increase in the weight of fry defects.

Producing a less polluted flow of combustion gases reduces the exposure of crop to contamination. Adopting chemically different fuels can alter the extent of contamination produced on burning. Even with the use of cleaner fuels, relying on hydrocarbon combustion to generate the hot air stream required will produce an impure fog. However the degree of contamination will principally depend on the fuel type. This is evident from the poor fry colour exhibited by all samples collected one day after the CIPC thermal-fog was applied.

Undoubtedly the impact on processing quality can be reduced by using a cleaner fuel than petrol.

The fry colours obtained when using methanol as a propellant were a big improvement on the samples treated with the current industry standard. This was expected as methanol has the smallest chemical structure of the fuels tested therefore it could only form a

comparatively limited range of compounds. Thus, the smallest range of volatiles of a contaminant nature are introduced to the storage atmosphere.

Improved fry colours were anticipated when LPG was used as a propellant for fogging. However the distinction between petrol and LPG was marginal. This was the first attempt at using LPG generated fog in crop trials with this equipment. In these circumstances it did not prove any more detrimental than petrol. The unexpected LPG results were probably due to an inefficient burner system, which meant fuel consumption was artificially high (*N. Green, personal communication*). This greater volume of fuel produced more combustion gases and contamination than would be the case if the system had been fully optimised.

Further development of an LPG fogging system is underway. This includes improving the combustion of LPG, finding the appropriate cylinder size and type and the necessary pressure control valve.

Trials planned for BPC project 235 based on the findings from this project should determine the true effect of an LPG propellant. Although *Gamas et al, 1999*, demonstrated that only a marginal improvement in emissions, including unburned hydrocarbons, was noted when LPG was compared with conventional automobile fuel.

Fuel consumption was measured for each fuel type with a Unifog machine under application conditions (chapter 2). This was compared with the relative cost of each fuel per unit volume. A table of properties associated with the fuels was produced (Table 60). These factors have to be weighted against each other to determine on balance how desirable the fuel is for use as a CIPC thermal-fog propellant.

Fuel properties	Methanol	LPG	Petrol
Carbon dioxide, water vapour & contaminant production	Low	Intermediate	High
Fuel consumption compared with petrol	High	High	Standard
Relative cost per unit volume	High	Low	Standard

Table 58 Summary of fuel properties

Although the improvement in fry colour was significant with a methanol propellant it would be an expensive option. The fuel consumption was high when compared to petrol. Methanol is more expensive to buy and a greater volume of fuel would have to be carried by the applicators. A license to carry and use alcohol for industrial purposes would be required and staff would need training to attain the license. The potential saving through increased crop acceptability needs to be assessed and balanced against the envisaged cost of a CIPC application.

Compared with petrol, LPG is cheaper and cleaner to run and could mean improved fry colours at an affordable price. The fuel consumption will be low and the cost of fuel relatively cheap. It is presently used for many industrial and domestic purposes and so is readily available and adaptable. Refill stations are becoming increasingly more widespread.

The LPG system needs further development to increase the efficiency of the burner and work toward this is underway. When the LPG fogging machines are ready for commercial use, it is likely that the operator will be required to become CORGI registered for health and safety reasons. The initial outlay associated with integrating LPG fogging systems would be an investment in a potentially effective method of maintaining good fry colour while preventing sprout growth. Therefore it is my opinion that even though the results thus far do not indicate that a lighter fry colour will be maintained if LPG is used it does indicate they will be no worse than using petrol. The day-to-day running costs would cheaper with LPG and therefore even in this current state it would seem like a sensible option.

Fuel blends could be tested to select an improved combustion process (less contaminants) at a lower price and therefore provide viable alternatives (*Asfar & Hamed, 1998, Allen & Watts, 2000*).

The use of cleaner fuels is rather a fundamental development. When costs, logistics and reduced crop losses through rejection from processing markets are evaluated it could be implemented to the benefit of many in the potato industry

7.1.2.7 Experiment 3: Dose Rates

The objective was to compare the effect of applying CIPC at different application rates on processing quality.

The application of CIPC as a thermal fog is considered a stress to potatoes in storage. The result of creating a stressful storage environment is the darkening of fry colour and potentially offsetting the effectiveness of the sprout suppressant action.

By altering the dose rate of CIPC per treatment, the number of applications required in a storage season could be reduced. Cutting back the number of treatments would decrease the frequency of contamination from entering stores, hence reducing the occurrence of stressful conditions.

The disruptive influence of the application was examined by comparison with more frequent applications to provide an equal dose of CIPC for the season.

The efficacy of application would have to be determined to ensure the benefit gained in quality is not at the cost of limiting sprout control. It is regarded that sprout control would not be limited by this procedure and may in fact be enhanced, however this issue has not been investigated as part of the experiment. The potential that exists for enhancing sprout control is relative to the frequent offsetting effect of fogging (stressing) on the quiescent state of the potatoes.

Method

Experimental work was conducted at SBEU starting in January 2002 and continued until May 2002. The crop used was cv. Saturna. It was held in 12 tonne stores at 10°C and 95% relative humidity. CIPC (50% w/v in methanol) was applied using a Swingfog machine. The treatments were as follows:

Half rate: 0.25l per application. Four applications conducted at 0, 4, 8 and 12 weeks of storage.

Full rate: 0.50l per application. Two applications conducted at 0 and 8 weeks of storage.

Double rate: 1.00l per application. One application conducted at week 0 of storage.

The full rate treatment was used as the control. It is the rate stated on the formulation label for application of CIPC with this methanol-based formulation. A full dose in 12 tonne stores is normally 0.5L of MSS 50M, equating to 21.25g of active ingredient per tonne.

The time taken to apply was different for each treatment. It took twice as long to apply the double rate as it did to apply the full rate, and correspondingly it took half the time to apply the half rate.

Consequently the stores received differing amounts of combustion products and contamination from the fogging process.

On each sampling occasion four replicate samples were collected from each store and assessed for quality. The samples consisted of 10kg trays containing approximately 30 tubers. The fry colour of samples from each treatment regime was assessed prior to all four application dates and seven days following all four of the applications. Therefore quality was evaluated at 0, 1, 4, 5, 8, 9, 12 and 13 weeks of storage.

Assessments encompassed fry colour, fry defects and headspace concentrations of C_2H_4 prior to ventilation.

7.1.2.8 Results and Discussion

The fry colour results presented are the mean values of each treatment encompassing all samples. Error bars display the mean value plus and minus one standard deviation.

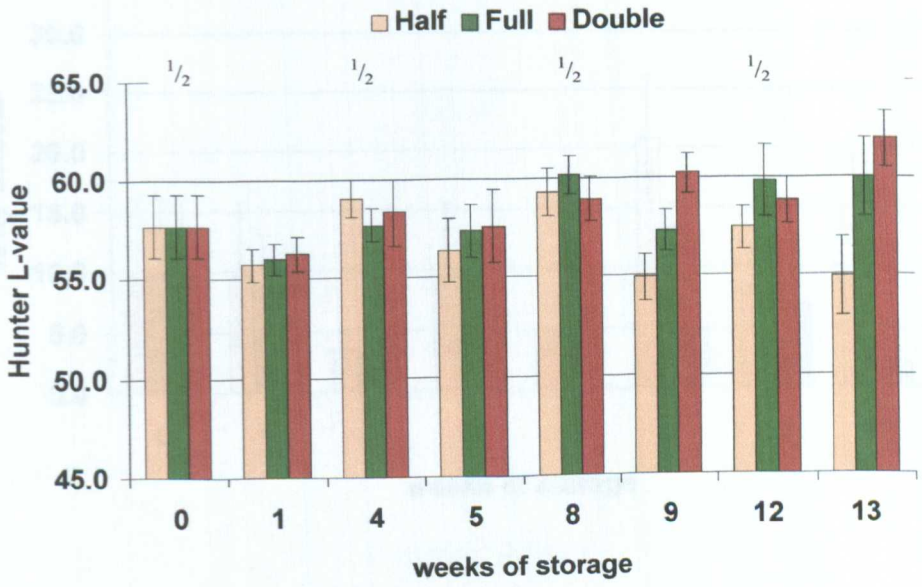


Figure 71 The effect of dose rate per application on fry colour over time (n=4)

Treatment	Wk 0	Wk 1	Wk 4	Wk 5	Wk 8	Wk 9	Wk 12	Wk 13
Half	a	a	a	a	a	a	a	a
Full	a	a	a	a	a	b	a	b
Double	a	a	a	a	a	c	a	b

Table 59 Statistical analysis of L-values. Different letters denote significant differences

As is illustrated in the above table all three treatment were significantly different on week 9. Half dose was significantly different from the full and double dose treatment on week 13. there were no other statistically different treatments.

Generally all fog treatments resulted in an initial deterioration in fry colour. However for the duration of the trial the samples with the darkest fry colour were those treated most frequently (half rate CIPC). Overall the samples with the lightest fry colours throughout were those only fogged once (double rate CIPC) at the start of the 13-week period.

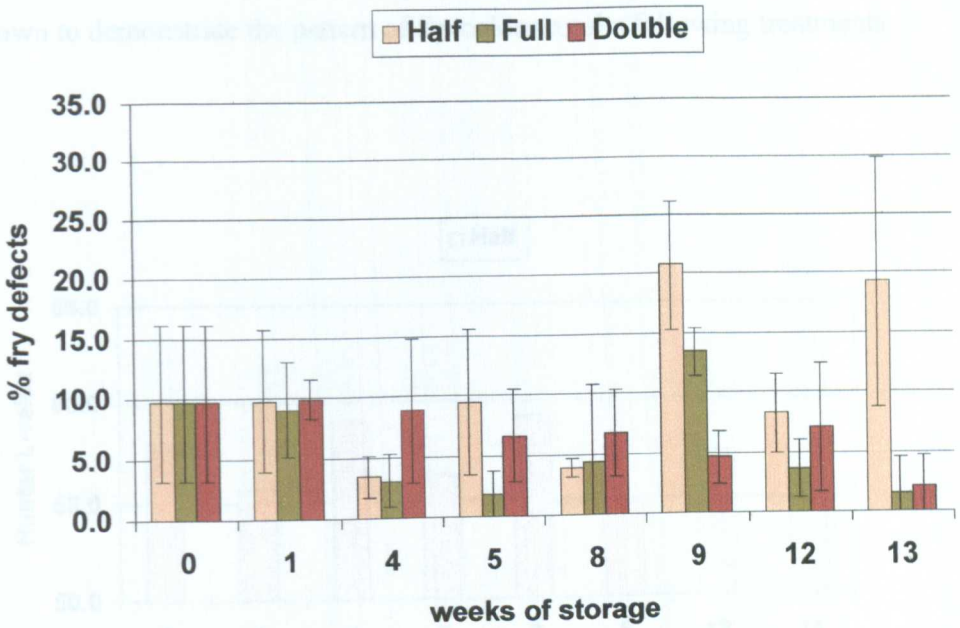


Figure 72 The effect of dose rate per application on fry defects over time (n=4)

Treatment	Wk 0	Wk 1	Wk 4	Wk 5	Wk 8	Wk 9	Wk 12	Wk 13
Half	a	a	a	a	a	a	a	a
Full	a	a	a	a	a	b	a	b
Double	a	a	a	a	a	c	a	b

Table 60 Statistical analysis of defects. Different letters denote significant differences

After an initial increase, the % fry defects declined and then remained low in samples treated with the double dose of CIPC.

In samples treated with the full rate, fry defects increased after both applications, however steadily declined between 1-4 weeks following fogging.

The % fry defects rose after each fog application in samples treated with a half dose. The effect became increasingly pronounced with successive applications.

The effect of individual treatments is more apparent from the following graphs. Each variable is presented separately in a graph of fry colour (Hunter L-value) against the storage time in weeks. Error bars have not been included, instead only the mean values are shown to demonstrate the pattern of fry colour results following treatments.

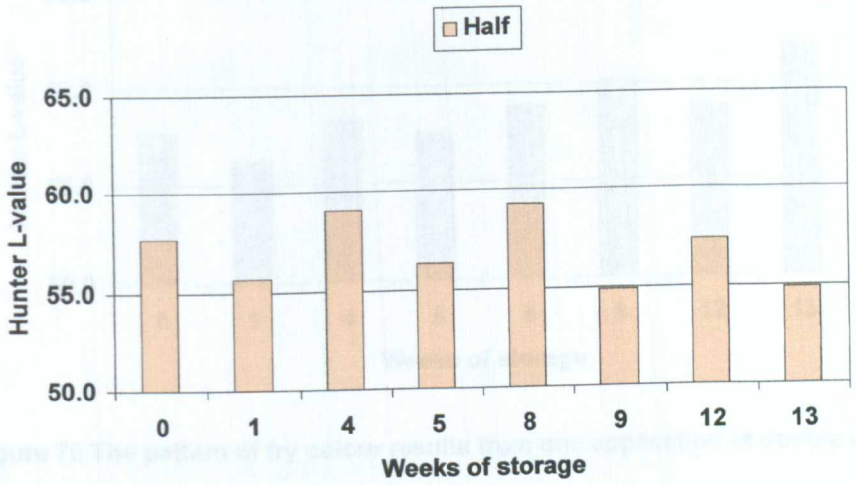


Figure 73 The pattern of fry colour results from four applications at half dose

From the half dose graph it is clear that each fog application caused a drop in quality. The repeated fogging means that after thirteen weeks of storage the crop was only in the lower end of the acceptable colour range for the commercial market (L value >58).

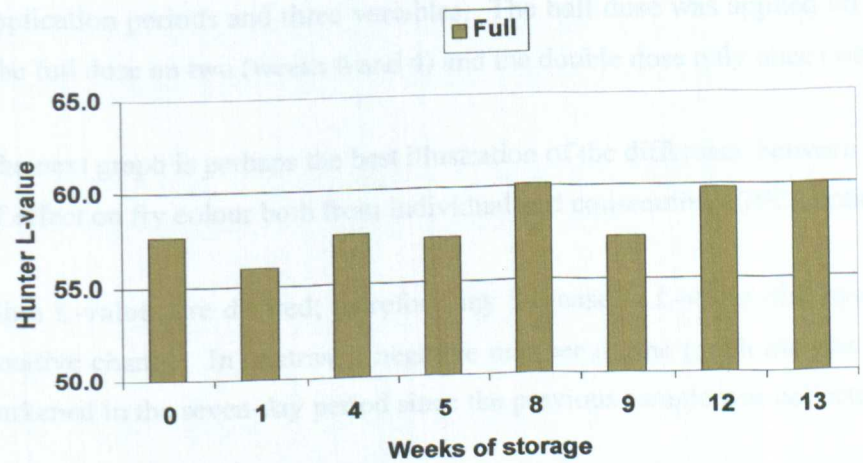


Figure 74 The pattern of fry colour results from two applications at full dose

With the samples fogged at full rate there was a similar pattern, however a longer period between applications allowed the fry colour to recover to some extent.

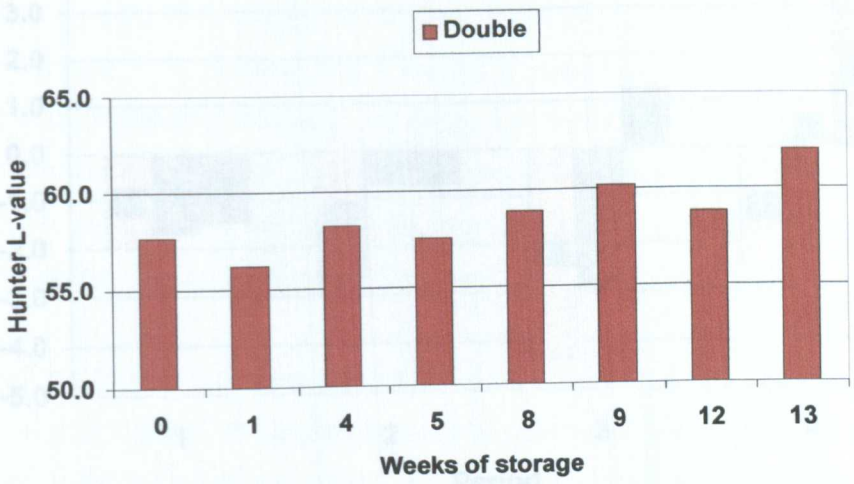


Figure 75 The pattern of fry colour results from one application at double dose

The samples fogged at double rate were the best quality. Cutting back on treatments and having more time between re-application appeared to improve fry colour.

The graph below is a plot of the difference between the L-value pre-application and seven days post-application. This information is included for every application period and variable. There were four application times (weeks 0, 4, 8 and 12) therefore four application periods and three variables. The half dose was applied on all four occasions. The full dose on two (weeks 0 and 4) and the double dose only once (week 0).

The next graph is perhaps the best illustration of the difference between treatments in terms of effect on fry colour both from individual and consecutive CIPC applications.

High L-values are desired; therefore any increase in L-value displayed in the graph is a positive change. In contrast a negative number on the graph dictates that fry colour has darkened in the seven-day period since the previous sample was collected.

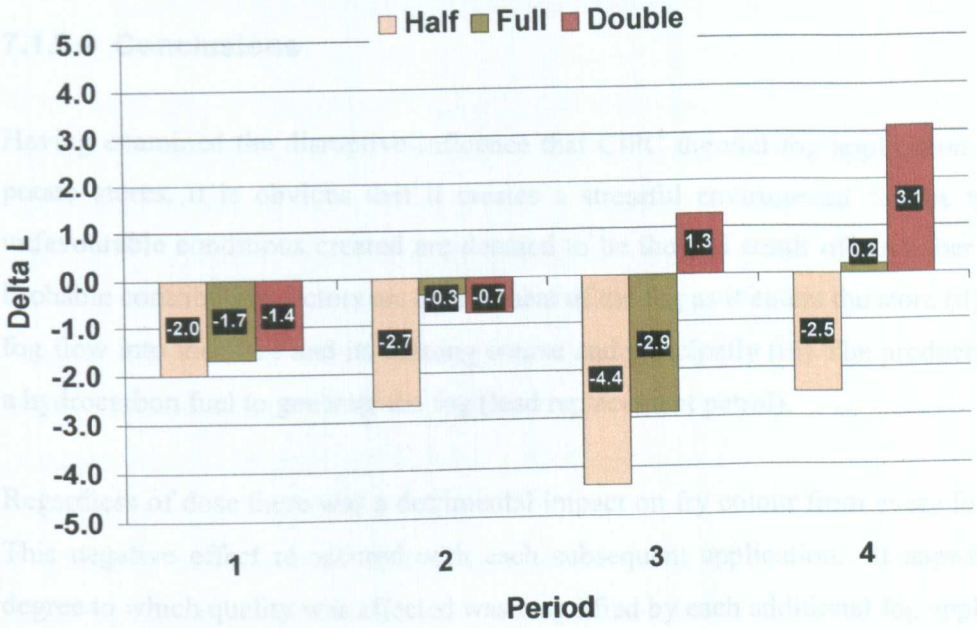


Figure 76 The difference in fry colour (L-units) between sampling occasions

The week following fog applications fry colour of samples had declined with every dose rate of CIPC. It was anticipated that the initial decline would be greater in samples that had been fogged for a longer time (double dose>full dose>half dose). However this was not the case. There did not appear to be much difference in fry colour response to treatments after the initial application.

Potatoes fogged most often endured the greatest stress. This was reflected in the large negative changes in fry colour of samples treated at half dose.

The frequency of fogging appeared to be a more dominant factor in influencing crop quality than the duration of the fog application itself.

The time taken to apply each dose did alter the overall amount of C_2H_4 detected in stores after treatment. The store treated with the double-dose, had the highest concentration at approximately 30ppm. The level determined in stores after full-dose application was within the range 10-30ppm on both sampling occasions. Correspondingly, in stores that received four half-dose treatments the C_2H_4 concentration was within the range 1-5ppm in each instance.

In these circumstances, where recurrent exposure is involved, the simple presence of C_2H_4 is more significant than its abundance.

7.1.2.9 Conclusions

Having examined the disruptive influence that CIPC thermal-fog application can have in potato stores, it is obvious that it creates a stressful environment for the tubers. The unfavourable conditions created are deemed to be the end result of a number of features. Probable contributing factors are (i) The heat of the fog as it enters the store (ii) The rate of fog flow into the store and its ensuing course and principally (iii) The products of burning a hydrocarbon fuel to generate the fog (lead replacement petrol).

Regardless of dose there was a detrimental impact on fry colour from every fog treatment. This negative effect re-occured with each subsequent application. It appeared that the degree to which quality was affected was magnified by each additional fog application.

Recurrent applications could counteract the sprout suppressant activity of the CIPC. All applications conducted as part of this experiment have introduced ethylene to the potato stores in appreciable amounts. It is known that short-term exposure can stimulate dormancy break. The exposure occurs during the period in which fog is allowed to settle after application before stores are ventilated. The fact that exposure to ethylene is intermittent could augment this counter effect. It would not render CIPC ineffective but it is a potential complication that is best avoided.

A sufficient time interval before re-treatment can allow the quality of the potatoes to recover to some extent from the stress caused. Tailoring the CIPC dose per treatment, and thus reducing the total number of applications necessary, can achieve this.

At present, altering the dose used is not in accordance with the normal procedure or the permitted label recommendations for dose rate application. Although the same total quantity of CIPC is applied in a season, according to currently common practice. The label on the product in use must be read for details of accepted practice.

The efficacy of CIPC in these experimental circumstances has not been determined.

7.1.3 Investigation of methods of removal

7.1.3.1 Experiment 4: Modifying Store Atmospheres

It has been shown that thermal fogging generates volatiles that have a negative influence on potatoes in storage. Even in the short time before ventilation, the exposure of tubers to these compounds can critically increase the reducing sugar content of the crop. Hence causing the darker fry colour of crisps when processed.

The objective was to identify a means of removing the contaminants present in thermal fogs. The intention is that once a store is fogged, the crop inside would not be exposed to as many harmful by-products as with the existing process.

Preliminary investigations of adsorbent and scrubbing substances revealed that certain factors were crucial for a successful system. Before the optimal set of conditions could be resolved the adsorbent had to be tailored to the practical application required. The most appropriate substance depends on the compounds of interest, the matrix and treatment conditions, notably the required contact time, regeneration and safety measures.

As this is a meticulous process, requirements were curtailed from all typical by-products of combustion to ethylene specifically. Its influence has been examined thoroughly. The options available for this included a dynamic flow-through sampling systems drawing store air over titanium dioxide with UV light to form carbon dioxide and water vapour (*Bio-KES Model 348*). This was deemed to be too expensive for practical implementation and was therefore not used in investigative studies. Also silver salts, specifically silver nitrate is known for its antagonistic effect on ethylene formation and responses (*Abeles, 1992, Tiainen, 1992, Evans & Batty, 1994).*

Tailoring the experiment to ethylene allowed for quantifiable and reproducible application of ethylene as a standard treatment to be conducted. It was applied in precise amounts at regular intervals to the headspace of all storage containers in equal concentrations.

The substances originally tested for efficiency of adsorption were silver nitrate (Johnson Matthey Chemicals Limited), washed bone charcoal (Brimac 20:60 mesh) and activated carbon (Norit PK 1-3, lab no. NC15983). All three substances proved ineffective in a static system. Silver nitrate and washed bone charcoal were unsuccessful when shaken intermittently within an ethylene-spiked atmosphere. The action of the activated carbon

was improved by use in the shaking arrangement. The adsorptive action of the activated carbon was further improved by integrating a second carbon trap (Carbosieve SIII 60:80 mesh, Supelco 10293) within a dynamic set up. Therefore a dual activated carbon trap in a dynamic assembly was the ethylene scrubber in the main experiment carried out.

Activated carbon is a carbonaceous adsorbent with a high internal porosity, and hence a large internal surface area. It consists mainly of elementary carbon in a graphite like structure. It can be produced by heat treatments (activation) of raw materials such as wood, coal and peat. The internal pore structure created during activation provides the exceptional adsorptive properties.

These carbon materials have a high affinity for a range of compounds, specifically carbon-based molecules and in particular those of low molecular weight. This makes them ideal for the purpose of the trial although they can be costly. The advantage in using carbon material is that it can be regenerated and therefore used repeatedly.

Another method of modifying the store atmosphere was creating elevated carbon dioxide levels in an attempt to inhibit the behaviour of ethylene.

A further tactic undertaken was to use an ethylene trap in conjunction with a carbon dioxide trap to try to remove both from the store air.

The crop quality was quantified by measuring the reducing sugar concentration of tuber samples.

Method

Experimental work was conducted at GU in 2l and 5l storage containers, each holding 2kg of cv. Saturna that had been treated with CIPC earlier in the season. The tuber temperature was maintained as close to 10°C as possible at all times, however minor fluctuations did occur. These fluctuations were reflected in the reducing sugar data. However, all samples were subject to these small changes and therefore any resultant trend was common to all treatments.

Each treatment was replicated three times, twice in the 2l containers and once in a 5l container. The smaller storage tanks were considered to be representative of typical store conditions. They were relatively closed systems yet some exchange with ambient air was

expected. They are referred to as 'leaky' stores. The larger storage tanks were totally closed systems allowing no exchange with ambient air other than when deliberately ventilated ('sealed' stores). All storage containers were ventilated frequently at suitable times arranged around treatment applications and sample times. The oxygen and carbon dioxide (from respiration) levels were never allowed to become restrictive. The containers were kept in a storeroom in which the temperature was controlled. Therefore any mixing with ambient air and ventilation was still within a controlled situation.

Ethylene applications were conducted using standard cylinders of pressurised gas (Scotty Gas) of two concentrations. The 100ppm gas was used at flow rates of 25ml/min and 10.5ml/min to create concentrations in containers of 10ppm and 4.2ppm respectively. A cylinder of 10ppm was used at a flow rate of 25ml/min to create a concentration of 3ppm. The treatments were applied on days 0 (4.2ppm), 7 (10ppm), 14 (3ppm) and 21 (3ppm) of storage.

Carbon dioxide was applied from a standard pressurised cylinder of 10% CO_2 in air (BOC gas). A flow rate of 25ml/min was used to create a 2% concentration in three specific containers.

Following application of the C_2H_4 and/or CO_2, the air inside the containers was circulated through the appropriate trap. The entire volume of each container (2l or 5l) was passed over the trap at a flow rate of 20ml/min using a peristaltic pump and PVC tubing.

The traps used were;

To remove C_2H_4 only: A headspace bottle with 1g activated carbon with a glass wool plug and a silanized glass tube packed with a 2cm bed of carbosieve SIII resin with glass wool plugs.

Elevated CO_2: Applied from cylinder to achieve a 2% concentration in container. Circulated only through the PVC tubing.

To remove C_2H_4 and CO_2: As above to remove C_2H_4, following these traps was a CO2 trap. It was a test tube with a side arm, rubber bung and inlet tube through the bung. It contained 20ml 2M NaOH, through which the container air was bubbled.

Control: circulated through the PVC tubing only.

The concentration of C_2H_4 remaining in store air 24-hours after treatment was determined to assess the % loss. 2.5ml disposable plastic syringes with 25G 1.5inch needles (luer lock) were used for collection. The syringes were plugged with a PTFE coated silicon bung until 2ml was injected into the GC for analysis approximately 2 minutes after collection. The conditions of GC used are detailed in the methods chapter using the PYE machine. The GC was calculated with standards of ethylene gas from pressurised cylinders. All injections were gas phase.

10ml and 25ml of store air was taken from the 2l and 5l containers respectively for measurement of CO_2 from respiration. These samples were collected 24-hours after treatment. The store air was bubbled through 2ml of 1M NaOH in small vials. The vials were then closed until analysed by colorimetric titration as outlined in the chapter 2.

Reducing sugar concentration was measured the day before each application, one day after and six days after the last C_2H_4 treatment. Two random tubers were collected from containers on every occasion and analysed for sugar content according to the methods described in chapter 2.

The air inside the containers was refreshed regularly by removing the lids and allowing exchange with the room air for at least one hour.

7.1.3.2 Results and Discussion

Reducing sugar concentrations are presented separately for the 2l and 5l containers to demonstrate the difference in efficiency of methods of modifying store atmospheres under entirely sealed and normal (slightly leaky) conditions.

The effect of intermittent ethylene exposure on reducing sugar concentration was more pronounced in the leaky containers. The extent of air exchange with ambient was dependent on the environmental conditions. The response of the modifications conducted was slightly different from that in the sealed containers, as the underlying impact of ethylene was greater. This was clear from the control treatment, where ethylene was applied, but no effort was made to remove or reduce its concentration.

The following graphs are the mean results of the reducing sugar concentrations of the samples stored in the 2l tanks. An asterisk indicates the sample was taken on the day

following ethylene/carbon dioxide application and circulation. Error bars are not included because it does not allow the data to be presented in this preferred fashion.

The fructose concentration was consistently lower than glucose in all samples from all treatments. There was no definitive ratio of fructose to glucose concentration, however in general both of these sugars show the same trends. There were occasional exceptions when one of the extract aliquots generated a nonsensical value that had to be discounted.

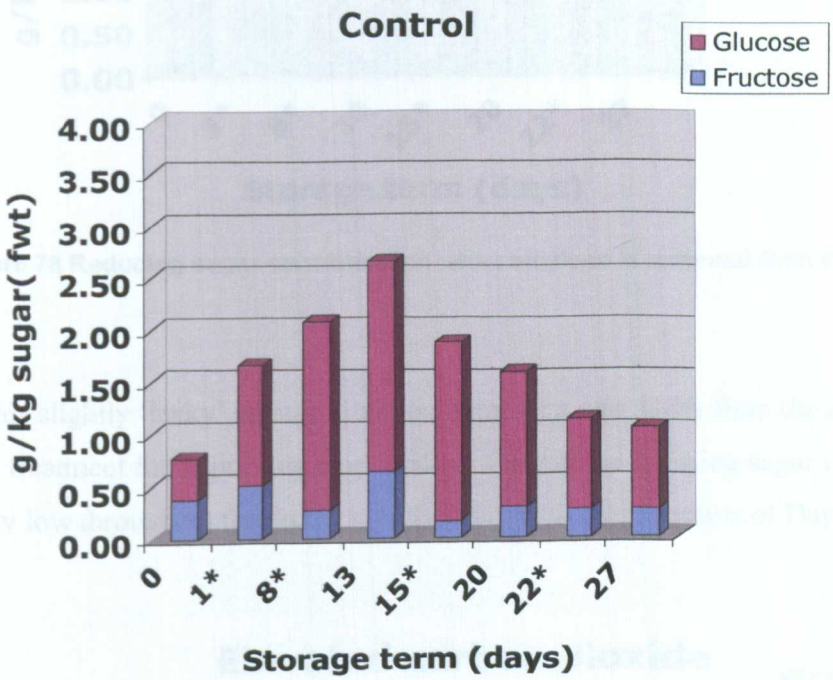


Figure 77 Reducing sugar concentration in the control samples over time (2l)

The control treatment showed quite clearly the detrimental effect of C_2H_4 exposure on the reducing sugar concentration. This was most pronounced on Day 13, where the result of prolonged exposure was evident, by the elevated level.

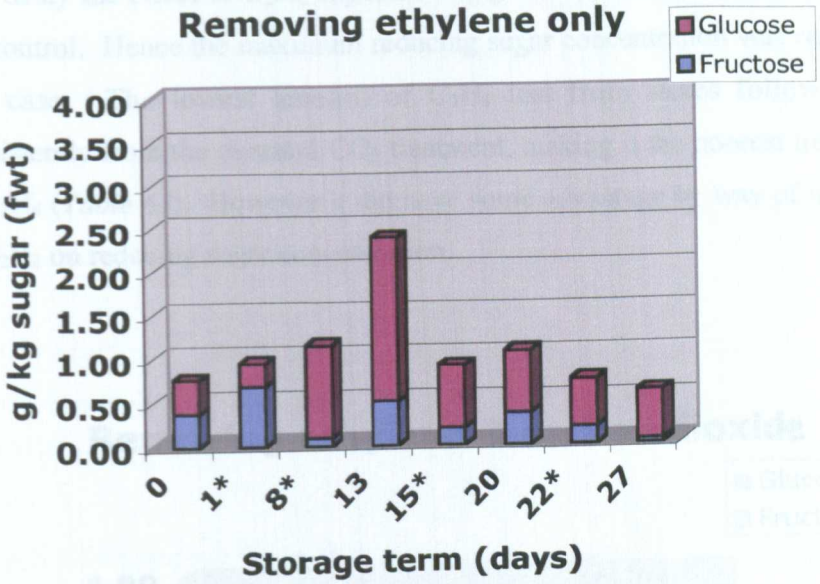


Figure 78 Reducing sugar concentration when ethylene is removed from the atmosphere (2l)

In this slightly 'leaky' storage situation, removing only C_2H_4 from the atmosphere was the best treatment for improving crop quality. Overall the reducing sugar concentrations were fairly low throughout the duration of the trial, with the exception of Day 13

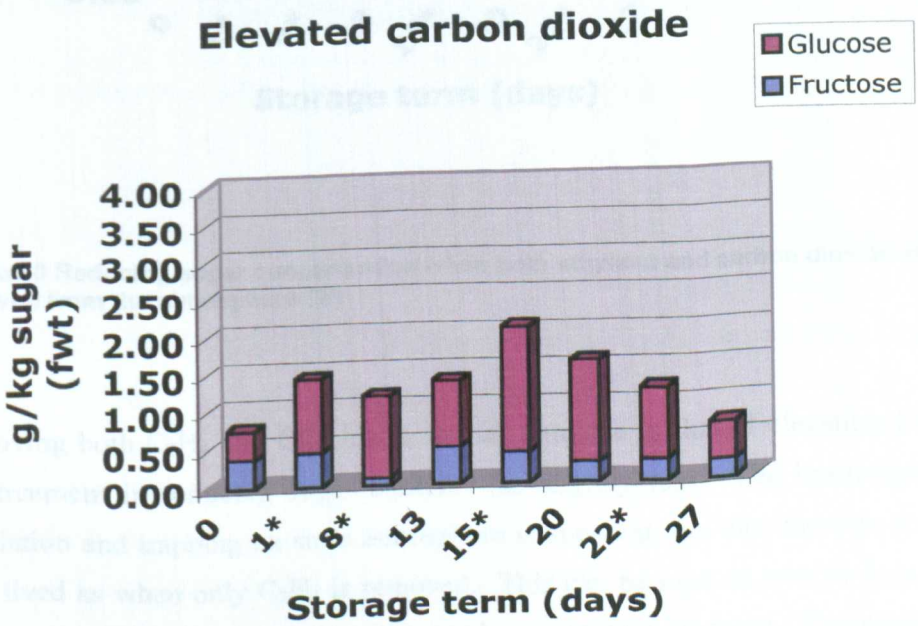


Figure 79 Reducing sugar concentration when an elevated concentration of carbon dioxide is created in the atmosphere (2l)

Creating elevated levels of CO_2 had some beneficial action. It appears that this treatment may delay the effect of C_2H_4 exposure along with reducing the impact when compared to the control. Hence the maximum reducing sugar concentration was reached on Day 15 in this case. The lowest amount of C_2H_4 lost from stores following treatment was consistently from the elevated CO_2 treatment, making it the poorest treatment for removal of C_2H_4 (*Table 63*). However it did have some advantage by way of inhibiting the effects of C_2H_4 on reducing sugar concentration.

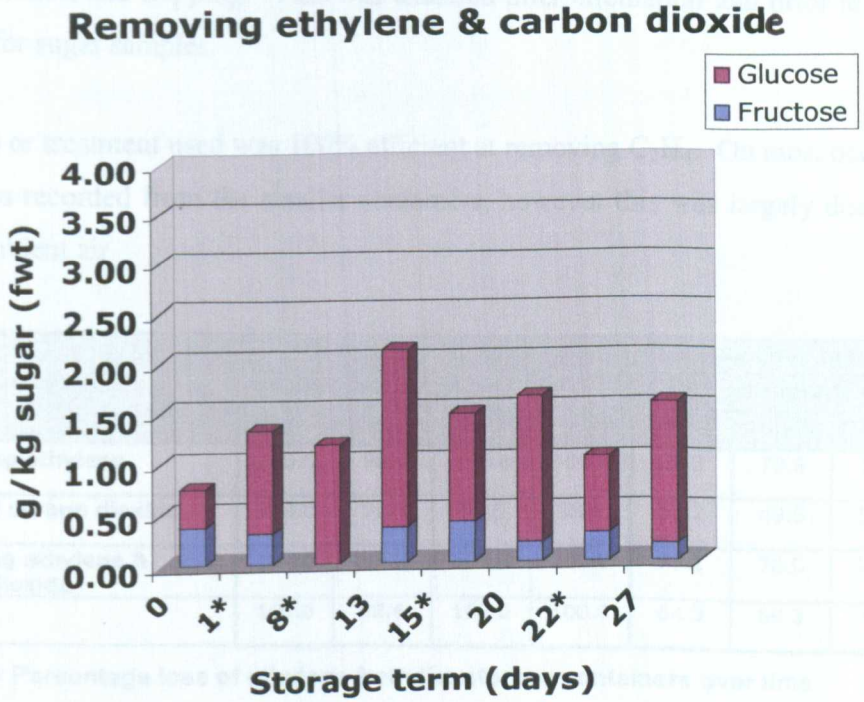


Figure 80 Reducing sugar concentration when both ethylene and carbon dioxide are removed from the atmosphere (2l)

Removing both C_2H_4 and CO_2 had a similar outcome to that of elevating CO_2, but the improvement in reducing sugar content was slightly less. The immediate effect of circulation and trapping on store atmosphere is apparent, but this improvement is not as long lived as when only C_2H_4 is removed. This can be seen on sample days 20 and 27 where levels have increased since the previous sampling occasion. The values for these two days do not follow C_2H_4 application and store air circulation/trapping.

This increased concentration on Day 13 was common to three of the treatments (Presence of C_2H_4 in store, Removing C_2H_4 from store and Removing both C_2H_4 and CO_2). It is believed to be the result of prolonged exposure to C_2H_4. The data recorded to monitor the loss of C_2H_4 from each container (*Table 63*) showed that on Day 8 local ambient conditions did not encourage sufficient air exchange to remove all residual C_2H_4. Consequently small amounts remained present in the storage containers for a longer period than following any of the other C_2H_4 applications.

The table (*Table 63*) below details the percentage loss of C_2H_4 from storage containers due to circulation and trapping. This was assessed after circulation and prior to collection of tubers for sugar samples.

No trap or treatment used was 100% efficient at removing C_2H_4. On most occasions 100% loss was recorded from the smaller containers, however this was largely due to exchange with ambient air.

Treatment	% loss of ethylene in twenty-four hours due to treatment							
	2 Litre storage containers				5 Litre storage containers			
	Day 1	Day 8	Day 15	Day 22	Day 1	Day 8	Day 15	Day 22
Removing ethylene	100.0	98.4	100.0	100.0	83.3	72.6	77.7	87.3
Elevated carbon dioxide	100.0	96.5	96.0	98.4	51.2	49.5	58.7	68.0
Removing ethylene & carbon dioxide	100.0	97.1	100.0	100.0	73.1	76.0	77.3	82.0
Control	100.0	98.6	100.0	100.0	64.3	58.3	72.0	78.0

Table 61 Percentage loss of ethylene from the storage containers over time

The system designed specifically to remove C_2H_4 was the most effective trap under both sets of conditions. Removing both C_2H_4 and CO_2 had some success but C_2H_4 traps were not as effective when coupled with CO_2 traps. In both instances the control lost more C_2H_4 from store than those containers treated with elevated CO_2.

Elevated CO_2 was not a method of C_2H_4 removal, but was a fairly successful method of countering the effects of C_2H_4 exposure on reducing sugar concentration. This system was effective in the smaller stores, but appeared to be limited in the 5l containers, which can be seen in the next few pages. It is possible that in these larger 'sealed' containers the levels of CO_2 were themselves affecting the reducing sugar concentration. For this treatment to be beneficial, adequate ventilation is essential.

Both sets of results in the above table have the same pattern, but the improvements in quality are greater in the smaller 'leaky' stores. This is partly because the effect of C_2H_4 exposure was more significant here and partly because the efficiency of the trapping systems was greater.

From the respiration rates (*Table 64*) it seems that the applied CO_2 was not present when the measurement was taken. This indicates it was not held in the store for long, but escaped. Even though its presence was transitory it had a notable effect on sugar levels.

The most steady respiration rate was that of the control samples, where the storage atmosphere was not altered (only circulated). This suggests the other treatments could have had a disruptive influence, causing the respiration rates to fluctuate, in some cases greatly.

Treatment	Respiration rate (mg carbon dioxide/kilogram/hour)							
	2 Litre storage containers				5 Litre storage containers			
	Day 1	Day 8	Day 15	Day 22	Day 1	Day 8	Day 15	Day 22
Removing ethylene	11.49	30.83	19.30	37.81	50.88	28.82	14.44	26.56
Elevated carbon dioxide	15.46	21.99	19.68	32.65	20.32	7.60	13.85	28.93
Removing ethylene & carbon dioxide	6.29	25.09	16.85	29.54	27.96	21.11	17.47	44.62
Control	20.35	20.62	21.31	22.06	35.60	33.08	16.60	37.19

Table 62 Respiration rate of samples in storage containers over time

The following graphs are the mean reducing sugar concentrations of the samples stored in the 5l containers. Again an asterisk indicates the sample was taken on the day following ethylene/carbon dioxide application and circulation.

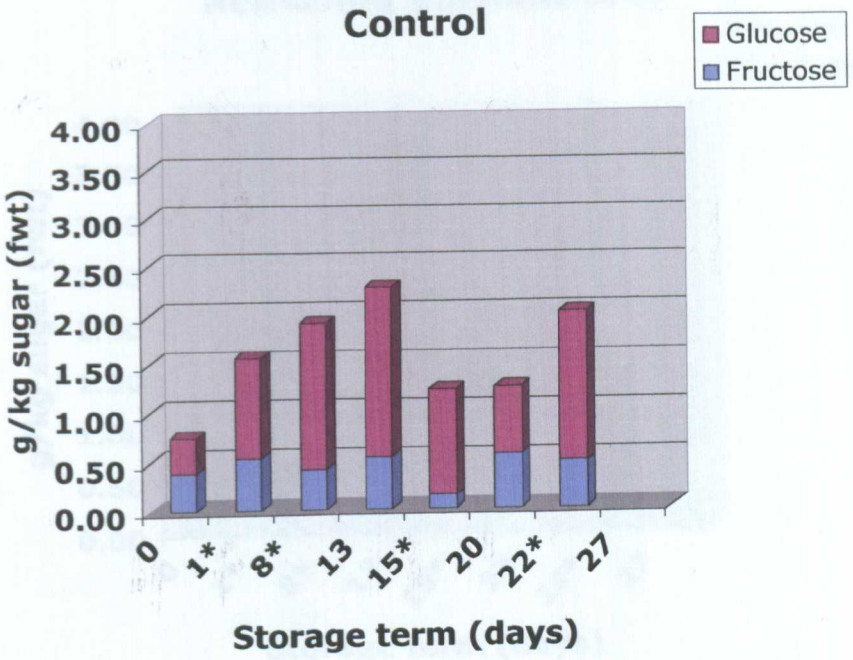


Figure 81 Reducing sugar concentration in the control samples over time (5l)

Again the control samples displayed the negative effect of C_2H_4 exposure on reducing sugar concentration. A similar pattern occured to that of the control samples in the smaller 'leaky' containers. However in the second half of the storage term the reducing sugar concentrations were lower in these 'sealed' containers. It seems the impact of C_2H_4 exposure was lessened in an environment where exchange with ambient air was non-existent (excepting intentional ventilation). It is possible that oxygen concentrations had become limiting and prevented the effects of ethylene being exemplified to their full potential.

Seemingly less damage occurred in these 5l containers where the only exchange with ambient air occurs during deliberate ventilation. Despite this the improvement generally by getting rid of ethylene from store atmospheres was more beneficial than constraining its effects by allowing oxygen levels to drop.

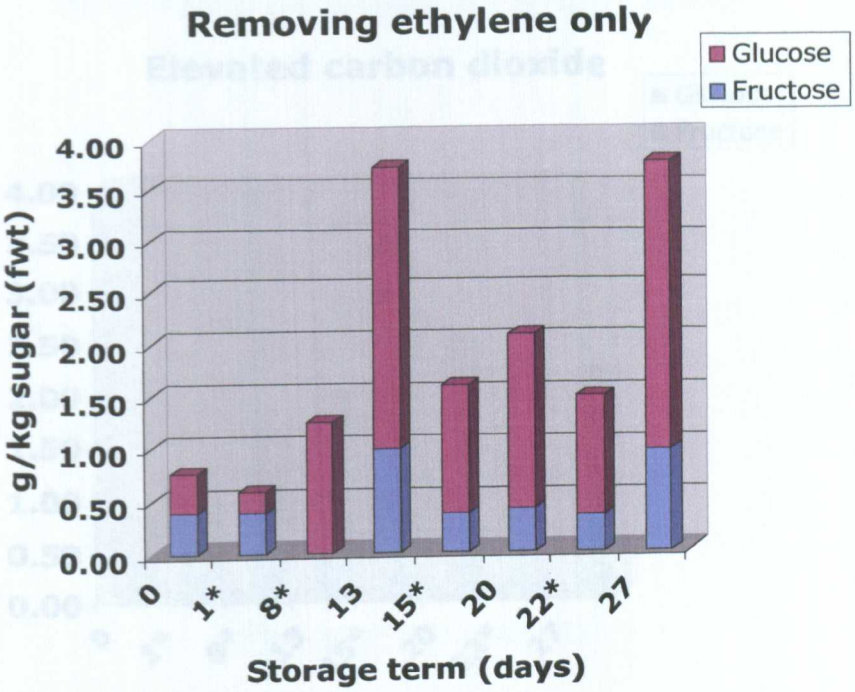


Figure 82 Reducing sugar concentration when ethylene is removed from the atmosphere (5l)

The outcome of the removal/inhibition of C_2H_4 was different in this situation. It was not as beneficial as it was in the 'leaky' environment. With the exception of day 27, the same general pattern of results was observed, but the levels here were on most occasions slightly higher. The traps were not nearly as effective in this situation, albeit unaided by the ambient air. When the impact of the C_2H_4 exposure was compared in both states of storage, the reduced effect in the 5l containers was most likely due to the availability of oxygen, influencing both the effect of C_2H_4 on sugars and the effectiveness of the traps to remove it.

On a number of sampling days the methods of removal/inhibition seem to be more detrimental than the exposure to C_2H_4 itself, mostly with removal of C_2H_4 and elevated CO_2 levels. This could be another example of the lag period noted in earlier work between the ethylene concentration in the store air and the resulting increase in reducing sugar levels.

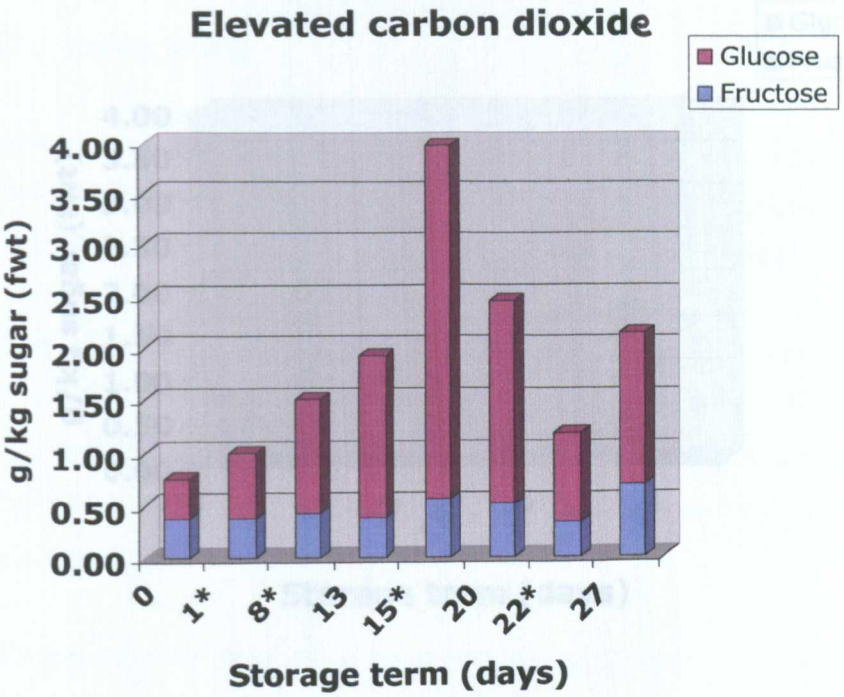


Figure 83 Reducing sugar concentration when carbon dioxide levels are elevated in the atmosphere (5l)

Elevating CO_2 levels was also not as advantageous in the 'sealed' atmosphere. Again the trend is comparable to the equivalent treatment in the smaller containers, but the reducing sugar values were generally higher. The glucose content appeared to be much more sensitive to changes in the ambient CO_2 levels than fructose.

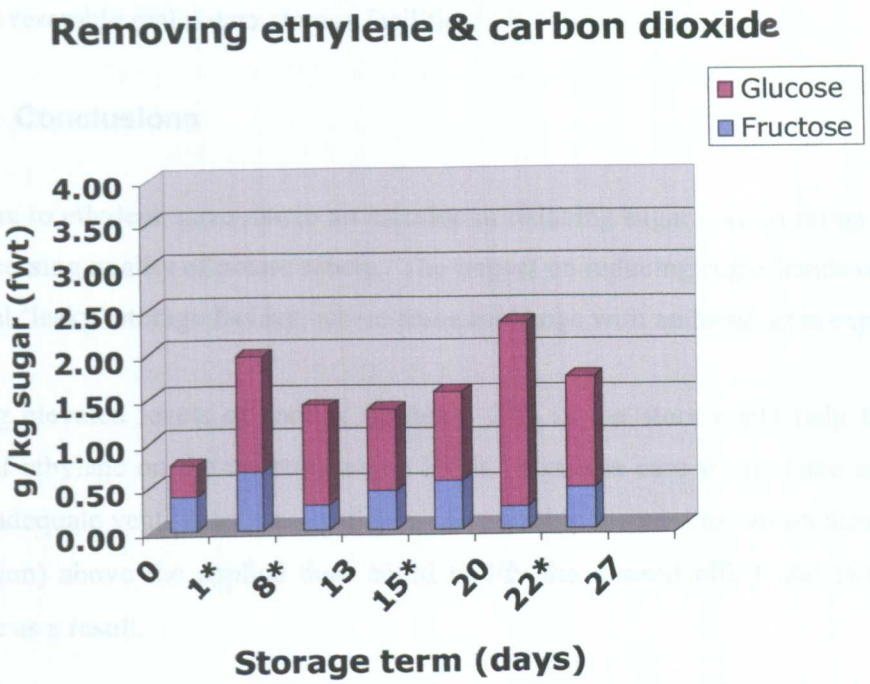


Figure 84 Reducing sugar concentration when ethylene and carbon dioxide was removed from the atmosphere (5l)

The result of removing both C_2H_4 and CO_2 was not as clear from the samples held in the 'sealed' storage containers. When compared with the smaller store values the pattern seems somewhat erratic. It is most similar to the control graph in the 'sealed' set up. The reducing sugar levels were lower than that of the control on only two occasions. This implies that the treatment also has a disruptive influence, but to a lesser extent.

Comparing all of these results with the control indicated that none of the treatments were constructive in a 'sealed' environment. Even combining two partly successful treatments (determined from the 'leaky' stores results) has no extra advantage if the conditions of use are not appropriate.

Overall, the reducing sugar concentrations resulting from the three methods employed in the 5l containers found that the most effective treatment in maintaining crop quality was to remove both C_2H_4 and CO_2. When odd values such as Day 13 (prolonged exposure) and Day 27 are excluded, the treatment of removing C_2H_4 only would provide crop of lower reducing sugar levels. This illustrates the extent to which ambient conditions can influence the storage environment and any procedures undertaken.

The smaller (2l) 'leaky' containers that undergo some unintentional exchange with ambient air most resemble real potato storage facilities.

7.1.3.3 Conclusions

Exposure to ethylene gave rise to an increase in reducing sugar concentration and lowered the processing quality of potato tubers. The impact on reducing sugar levels was greater in a normal 'leaky' storage facility, where some exchange with ambient air is expected.

Creating elevated levels of carbon dioxide (~2%) in the store could help to inhibit the effect of ethylene on the reducing sugar levels. Extreme care would have to be taken to ensure adequate ventilation was available. Any sizable increase in carbon dioxide (through respiration) above the applied dose could nullify the desired effect and cause sugars to increase as a result.

Removing ethylene from the store atmosphere, by means of an adsorbent material, to reduce the exposure time can be beneficial in preserving crop quality.

Those adsorbent systems tested (activated charcoal and Carbosieve SIII resin) were not 100% successful in scrubbing ethylene from the storage atmosphere. The efficacy was heavily dependent upon the local environmental conditions. The traps were much more effective in a 'leaky' situation (even with the greater impact of ethylene exposure) than in a closed system without any exchange with ambient air.

The gas balance and most likely the oxygen availability seem to be important in the performance of the removing/inhibiting of ethylene action on the potatoes. Achieving and sustaining the ambient conditions required is imperative to the success of the adsorbent system.

Ideally the adsorbent system would be as close to 100% efficiency as possible. The adsorbents examined were highly receptive to ethylene and are good options for incorporating into an improved system. The key feature that remains to be addressed is determining the simplest and most practical means of passing the store air over/through an adsorbent. The optimal operational procedure for doing this would also have to be assessed (including air flow-rate and regeneration).

This is a functional option for minimizing the effect of hydrocarbon fuelled thermal fog application of CIPC on the processing quality of stored potatoes. The benefit of the treatment in a large commercial potato store and any effect on CIPC efficacy has yet to be established.

Another option with potential for minimizing the effect of thermal fogging on fry colour is to fit catalytic converters on fogging machines to reduce the production of harmful volatiles. Also scrubber system could be used, employing activated carbon in some form, in a dynamic system with well established operating conditions and regeneration times. These types of systems would have been costly and time consuming to investigate within the time scale of this study. The approaches which have been adopted and described in this chapter are more immediately applicable to commercial potato storage.

7.1.4 Conclusions

Baseline fry colours have been shown to be different depending on the duration of storage and cultivar (*Agblor & Scanlon, 2002*). This helps to explain the differences observed between experiments timed in May and November.

The apparent heightened sensitivity of the crop later in the storage season could be partly responsible for the more obvious differences noted between treatment effects. For example in May when the crop is older and has been subjected to storage conditions and previous applications of CIPC small differences in ethylene concentration and exposure time may have a greater effect on fry colour i.e Methanol was more of an improvement when compared to petrol in May as against the comparison in November when the crop was younger. This has to be considered when interpreting the results of storage trials examining different treatment effects.

On the whole the attempts made at reducing the negative effect of thermal fog application on processing quality have been relatively successful. The trends are quite clear. An improvement in Hunter L-values of only 2 or 3 points is important for processing end use.

The greatest improvement was obtained from early ventilation of stores following fogging, thereby reducing the exposure time of potatoes to combustion products and especially ethylene. Further improvements might well be possible by combining the use of a cleaner fuel to generate the fog with early ventilation. This scheme would be fairly easy to integrate with current practice and does not require major changes to be made in stores. As a practical application of simplicity and low-cost it is an attractive and logical tactic for maintaining processing quality in conjunction with sprout control. Methanol provided crisps of the lightest fry colour and is the best option so far within this small range of hydrocarbon fuels.

Ideally the reliance on hydrocarbon fuels will decline and the use of heat exchangers to provide an air stream at an appropriately high temperature may become commonplace. The larger applicators in the UK are individually working towards this objective, but it is a slow and costly process to produce a machine that is effective in applying CIPC with reduced impact on quality and that can be readily powered on site and transported between sites without excessive cost.

Tailoring dose rates of CIPC shows promise for both reducing the overall amount of pesticide applied from a residue point of view and cutting back on the frequency of exposure of potatoes to the by-products of thermal fogging. The efficacy of sprout control resulting from such a regime remains to be seen. This issue would probably be the principal concern of store managers who need to achieve and maintain good control for the entire storage season and undoubtedly believe that regular treatment is essential. Even now with numerous applications throughout storage, achieving effective sprout suppression is a hard task.

8 Chapter Eight

8.1 FURTHER RELEVANT INVESTIGATIONS INTO POTATO CHARACTERISTICS AND BEHAVIOUR

The following section covers work done towards the end of the three years of practical experimentation. These areas were considered relevant to the project as a whole and necessary for completing a rounded piece of research on this topic. However they do not pertain exactly to individual chapters of work presented and are therefore included as a group in this separate chapter.

One of the core objectives of this project and related studies has been to maximise the sprout control ability of the CIPC applied and therefore reduce the total used per season. This takes in aspects of quality, cost, safety and environmental concerns. From a quality perspective any stress exerted on stored potatoes is detrimental. Some of the effects of undue stress have been presented throughout this thesis, principally the negative impact of thermal fog application on reducing sugar levels and subsequently fry colour. The underlying cause of the drop in processing quality following CIPC treatment is the presence of ethylene as a contaminant in the fog. Obviously methods of avoiding or negating this problem are required.

Only applying CIPC when it is needed sounds like a simple concept, however it is very rarely what actually happens in practise. Postponing a treatment until it is absolutely necessary carries a risk. It is difficult to know exactly when CIPC should be applied to be most effective in curbing sprout growth. Waiting too long can be costly and not being able to plan for an application too far in advance is inconvenient for store managers. If a system could be developed that would indicate when the potatoes are nearing the end of their natural dormancy period, without causing disturbance, it could be of enormous benefit.

Changes in the pattern of naturally occurring potato volatiles happen at physiologically significant events and in response to stress as discussed in the following pages. If these changes were to be monitored and classified, they could also be used as predictive tools for indicating for example when the onset of sprouting is imminent and hence when CIPC needs to be applied. An attempt was made at building a picture of volatile events in the experiments overleaf.

8.1.1 Potato volatiles

The volatiles exuded from potatoes have been of interest for many years, mostly as an indication of what chemicals could potentially be used to alter the behaviour of the potatoes in storage with a view to controlling sprout growth (*Burton, 1952, Meigh, 1969, Varnes, 1979, Vaughn & Spencer, 1993*). As part of this project an experiment was undertaken to try to identify the key volatiles produced by crop at particular stages of development. This is of interest because volatile compounds are produced in differing concentrations as the crop ages. The main concern of this project was to identify those volatiles that peak in concentration immediately prior to the opening of eyes and the onset of sprout growth. If this was possible then perhaps the information could be used to design sensors that would indicate to store managers when the crop needed CIPC treatment, eliminating the necessity for application on a calendar basis. Earlier research funded by the BPC (*N.M. Radcliffe, Project 807/172*) working on early warning systems for disease detection in stored potatoes developed sensors that were activated by volatile concentration in the store headspace.

The volatiles of raw potatoes are cultivar dependant and can be influenced by the history of the crop e.g. fertilizer type. *Maga, 1994* compiled an extensive but by no means exhaustive list of volatiles exuded from raw potatoes. These could be subdivided into fifteen broad classification groups for chemicals: aldehydes, esters, ketones, alcohols, acids, hydrocarbons, pyrazines, furans, amines, ethers, halogens, thiazoles, miscellaneous sulphur compounds and general miscellaneous compounds. The profile of potato volatiles is different before and after cooking, and the pattern of compounds present in raw tubers contributes significantly to the flavour of cooked potatoes (*Oruna-Concha et al, 2001).*

Much of the research in this are has been concentrated on disease volatiles in an attempt to develop sophisticated detection systems (*Varns & Glynn, 1979, Waterer &.Pritchard, 1984, Schutz et al, 1996, Weissbecker et al, 1997, Schutz et al, 1999, Kushalappa, 2002).* The type of sensors that are being developed are gas sensing systems or 'electronic noses' that will signal when action or preventative measures are needed (*Craven et al, 1996, Amrani et al, 1997*).

Over the years methods of air sampling for volatiles (including potato store sampling) have been improved but they still centre around the essential stage of passing headspace gas over or through a substance that will retain the analytes of interest for a period long enough for an adequate concentration to accumulate (*Bertsch et al, 1974, Black et al, 1977, Filmer*

& Land, 1978, Filmer & Rhodes, 1985, Lyew et al, 1999, Huxham & Thomas, 2000). The higher concentration of sample makes quantification more straightforward but the removal of the sample from the trap can be difficult. This is usually where the individuals modify procedures for the specific requirements of analysis of the sample. A particularly common adsorbent resin with a wide range of applications is Tenax (details given in chapter 2). This was the material used by *Radcliffe, 1999* for collecting potato volatiles in a laboratory environment. The method outlined in his project was followed as closely as possible because the nature of the work was very similar.

GCMS is the predominant method of analysis for volatile separation and identification. On the whole it works exceptionally well providing the sample format is compatible with the equipment (*www.zymaxusa.com/technotes/tph-measure.html, 2000, Zoccolillo et al, 2000*). This usually means liquid solvent samples, which are preferred for GC analysis. Thermal desorption can be an excellent way of quantifying sample of very low concentration because the dilution step (solvent elution) can be avoided. Where samples need to be collected on resin and no facility for thermal desorption is fitted to the GCMS analysis becomes more complicated, and ways around this problem have to be developed. GC-FID has been used successfully for *in situ* determination of hydrocarbons in air (*Konrad & Volz-Thomas, 2000*). Unfortunately the demands in terms of sample preparation are the same before useful analysis can be conducted.

8.1.2 GCMS work on volatiles

The aim was to collect a selection of main potato volatiles and quantify these with a view to comparing their relative proportions under different levels of stress.

8.1.2.1 Design and Methods

2.9kg (26 tubers) of cultivar Saturna were placed in a 5l dessicator and the lid was sealed around the edges with PTFE tape. In the top of the lid there was a PTFE coated bung with two silanised glass tubes (outside diameter 6mm, inside diameter 3mm) inserted through it. The tubes were sealed during storage but were later used to connect tubing for air sample collection. The dessicator was held at room temperature.

192g of diced Saturna tubers were placed into a separate smaller dessicator (0.5l). The dessicator was sealed and stored at room temperature. It had an identical bung with two glass tubes inserted through it.

Only two individual set ups were used as the method was new and the intention was to develop a functional and reproducible technique before more extensive experiments were carried out.

The sealed and relatively high temperature storage conditions were used to encourage volatile production, particularly those that would normally be expected prior to initiation of sprouting and some other common comparatively abundant compounds. In the case of the diced potato the intention was to allow a build up of defence volatiles normally produced during would healing and periods of stress. The volatiles of interest were a range of hydrocarbons, aromatics, alcohols, ketones, aldehydes and napthalenes. The list of standards included is given in the following *Table 65.*

Compound group	Name
Hydrocarbons	Ethylene
Aromatics	Benzene Ethylbenzene
Alcohols	Methanol Ethanol Butanol
Ketones	Acetone
Aldehydes	Acetaldehyde Benzaldehyde Hexanal
Naphthalenes	Napthalene Dimethylnapthlene

Table 63 List of volatiles of interest evolved by potatoes in storage

The glass sample tubes (6mm outside diameter, 3mm inside diameter) had been previously silanized, then packed with a 15mm bed of absorbent resin (Tenax –TA) and a silanized glass wool plug at either end. All sample tubes were conditioned prior to use.

The samples were collected twenty-four hours after the dessicators were sealed. An air-sampling pump was connected to each sample tube, which was then connected to one of the glass tubes inserted through the bung. 3l of the dessicator air was drawn over the resin

at a rate of 50ml/min. After 5 minutes of sampling the second tube through the bung had the seal removed and was covered with teflon tape with a small puncture in it. This was to allow airflow through the dessicator without any build up of pressure. During sampling from the smaller dessicator the opening of the second tube through the bung was earlier at only one minute after sampling started because of the smaller total volume of the storage container.

After sampling the ends of the glass tubes were sealed with PTFE tape and aluminium foil. The samples were immediately taken to a GC Mass Spectroscopy (GCMS) instrument for analysis. The conditions of the GCMS were as follows:

Column: Fused silica capillary column, 30m length, 0.32mm inside diameter, 4mm film thickness

Sample tube: heated to 200°C in injection port, with carrier flowing through

Cryo-trap: 5cm loop of column held in liquid nitrogen for 10 minutes while sample is desorbing (after ten minutes the trap is removed)

Oven: upon removal of cryo-trap oven is held at 40°C for 2 minutes, then heated at a rate of 4°C/min for 5 minutes up to 200°C and maintained at this temperature until the end of the run

Split/splitless: Ratio of 80% on to column, 20% to waste

Scan range: 10-300 atomic mass units

The first sample did not work. It caused the detector to overload and shutdown to prevent damage.

After discussing this problem with the experienced GCMS technician suggestions were made as to how to improve the chances of getting a result with the second sample

The suggestions were to:

Alter the split/splitless ratio to put less onto the column

Spike a resin tube with standards to check the method

Heat the injector port (resin) to a higher temperature to ensure the volatiles come off the resin

For the second sample only 5% was put onto the column and 95%was vented to waste. The detector appeared to be overloaded with this ratio also and shutdown immediately.

To test the method a blank sample tube was run through the system under the same conditions. This meant that if the problem was the detector overloading with volatiles released from the resin the blank sample would run without fault as there were no volatiles to be released. Unfortunately the blank sample did not work either. The same fault occurred leading to the shutdown of the detector. Clearly there was something fundamentally wrong with the method that was preventing a sample of this nature to be run through the system as normal.

The initial holding of the oven at 40°C was to allow any moisture to come through the column first before any expected volatiles and prevent masking or interference of peaks (even though Tenax-TA should not hold moisture, it was essential to make certain that even trace amounts be removed). The 2 minutes allowed for this was considered adequate for even a large amount of moisture. In the Earlier BPC work (*Radcliffe, 1999*) the hold at 40°C was 2 minutes and for the same reason. This was sufficient to stop problems associated with water and allow normal operation to continue. Various time delays were tried, both shorter and longer than 2 minutes, to determine if this could have been the source of the fault, however all of the times attempted lead to the detector shutting down.

This type of sample had not been attempted before in the GCMS equipment used at GU. The common sample form is a liquid extract, which is injected directly onto the column through the port. The problem most likely arose from trying to desorb the volatiles from the resin and successfully transfer a good proportion onto the column. The system was such that no leakage should have occurred and therefore no disturbance to carrier flow was expected. Whether this happened is unknown, but it was a potential point of weakness in

the system. The injection port had to be altered to fit the sample tube inside it; it was not a custom built design.

This unsuccessful attempt to identify the volatiles by GCMS led to the decision to try to desorb the resin in a conventional GC, separate the volatiles and identify by comparison with standards using Flame Ionisation Detection (FID).

A non-polar Megabore column (49188), 0.53mm inside diameter and 1.5µm film thickness which separates on boiling point only was put into the GC oven. A splitless liner was inserted and to check the column was working well an injection of butane was put through the column. The butane is not retained on the column at all and so passes through at the rate of the carrier gas (approximately 7.7ml/min).

Individual standards were prepared, in hexane, for each of the volatiles of interest at a concentration of 1000ppm. From these, individual 100ppm, 10ppm standards and a mixed standard of 100ppm of each compound were prepared in hexane. Under the following conditions the standards were run through successfully and the mixed standard could be separated well. Injector at 200°C, detector at 250°C, oven at 40°C for 10 minutes, then increased at 4°C/min to 200°C. The peaks were identified by comparison with the individual standards run under the same conditions.

The next step was to spike absorbent resin (Tenax-TA) sample tubes with a small amount of the 100ppm mixed standard and attempt to desorb and separate on this megabore column. 5µl of the mixed 100ppm standard was injected onto the top of the resin and with the aid of a 1ml plastic syringe (and some silicon tubing to connect on) it was pulled onto the resin until it was clear that the length of the resin bed was wetted. After spiking the tubes were sealed with teflon tape and left to equilibrate at room temperature for at least four hours.

Once equilibrated the spiked tube was inserted into the injection port and connected into the top of the liner. The injection port was heated to 200°C after insertion. A 5cm loop of the column was placed inside a liquid nitrogen trap simultaneously as the tube was connected and the carrier gas was switched on. The volatiles on the resin were desorbed because of the increasing temperature and brought onto the start of the column by the carrier gas. They were held on the section of the column submerged in the cryo-trap. The trap was in place for ten minutes. This allowed adequate time for all of the compounds to be desorbed. The larger, heavier volatiles would require more time until a higher

temperature was reached before being released from the resin. Using a cryo-focusing technique meant that all of the compounds could be released onto the column at the same time to prevent the prolonged desorption from the resin causing problems with peak definition.

Injections of the mixed 100ppm standard in hexane were also done using the cryo-focusing method to ensure appropriate comparison for identification was made.

8.1.2.2 Results and Discussion

Separation of compounds from the resin was not possible. The output from the GC was a high signal for a period of approximately 10 minutes, which masked any peaks that may have been present, although even that was not likely. Lesser spike volumes were prepared on resin samples and analysis attempted when the baseline had fully recovered. Again these attempts were unsuccessful and hence the problem did not seem to be overloading the column with analytes.

It appears that the same problem associated with thermal desorption of resin samples in a conventional GC (through the injection port) occurred with both machines used (GC-FID and GCMS).

The cryo-focusing technique was successful as demonstrated by the satisfactory separation and identification of the mixed standard sample.

Regrettably there was no further time available to continue working on this method and the attempt to collect and identify the selection of volatiles ended here.

8.1.3 Crop condition trial

Trials investigating the ventilation time post fogging (Chapter 7) found that the crop was more sensitive to treatments toward the end of the storage season when it was physiologically older. The status of the crop at this stage is largely a result of storage conditions and the effectiveness of sprout control (also dependent on inherent properties of individual cultivars). There is potential for an additional negative impact to have taken hold by this stage of storage. Repeated CIPC thermal fog applications give way to intermittent short-term exposure to ethylene in the concentration range 1-5ppm. Therefore stored crop at this time of year may not be as quiescent as desired.

To clarify whether this heightened response was common to other stresses exerted on the potatoes an experiment was conducted where potatoes were subject to non-ethylene related stress. The stress applied was intended to be representative of hot air being blasted (high flow rate) into a potato store (similar to a fogging situation). The key variable was the starting condition of the crop.

Two extremes of crop condition were used to illustrate the effect that the physiological state of the crop can have on its response to stress. All crop used was cultivar Saturna lifted at the same time from the one source and had been in storage in the same facility for an equal duration. Two treatments were imposed to allow ageing to occur in half of the potatoes. The crop referred to as 'good' was maintained under suitable storage conditions (10°C) until used for this experiment. The crop referred to as 'old' was kept in an incubator at 10°C but received different treatments. It had been previously exposed to elevated levels of carbon dioxide (approximately 1.5%) or ethylene (2ppm). It was mixed and stored together for at least ten days before use.

To enable the experiment the crop was exposed to stressful conditions of high temperature similar to that of thermal fog as it exits the application pipe upon entering potato stores. Although measurements made during this project has shown that at the end of a 7 metre aluminium-ducting pipe the temperature of air is still in excess of 200°C when the fogger machine is operated under standard running conditions (Chapter 4). Those in industry consider that fog temperature upon entering a store is below 100°C. It is not clear if this belief arises from practical experiments carried out in the past or if it is an assumption based on their experience of thermal fogging over the years. It is possible that the formulation solvent has a cooling effect on the air stream, but the extent of cooling would have to be examined. It might be the case that fog was below 100°C when older

equipment was used. Progressive developments in technology have been incorporated and perhaps this aspect has not been re-examined. Nonetheless the ostensible stress of the tubers resulting from exposure to temperatures in the region of 100°C is important to grasp.

In this experiment there was no ethylene involved. The effect being simulated is that of elevated temperature introduced as a dynamic flow through the storage facility. The time of application was scaled down to be applicable in 18l cardboard boxes. The aim was to create a short blast of hot air forced into boxes at a reasonably fast rate. The two stresses exerted on tubers from this treatment were the increased temperature and the force of the air movement around the container.

8.1.3.1 Design and Methods

5kg of Saturna was placed inside each 18l box. There were two treatments and three application variables each with three replicates. A carbon dioxide trap was put inside each box before lids were fitted. The boxes were stored in an incubator at 10°C. The crop was left in this condition for three days prior to treatment to settle.

Immediately before applications the lids of the jars containing the carbon dioxide traps were removed, the box lids replaced and sealed around the edges with parcel tape. The existing spaces at the corners of the lid were left open. This meant each box had an equal amount of leakage and ventilation and prevented a build up of pressure when the air stream was introduced. None of the adhesive on the tape was exposed. This was intentional to prevent any contamination or interference of results from Volatile Organic Carbons (VOC's) arising from the tape. An access hatch was cut into the long side of each box to allow a glass tube (outside diameter 6mm) containing adsorbent resin (Carbosieve SIII) to be inserted easily. This sample tube was inserted immediately prior to the commencement of treatment and removed one minute after treatment was completed. The hatch was sealed over directly after the tube was removed.

In the centre of the short side of the box, toward the bottom an insertion was made and the surrounding card cut to permit easy insertion of the metal nozzle of an industrial hair dryer. Again there was an access hatch over this opening for ease of opening and sealing when required.

The applications were identical for all six boxes. The hairdryer was used to create the hot rapid airflow, without the production of ethylene. Cold air was drawn into the back of the unit and heated as it passed a hot electrical element. The temperature of air exiting the nozzle was found to be in the region of 88-89°C when hot and 17-18°C when unheated. A cold air treatment was included also.

The flow rate of air out of the hair dryer was calculated from measurements of air speed, taken using an anemometer (Airflow TA-5 Thermal anemometer), and the cross sectional area of the circular exit port of the unit. This had to be done with unheated air that was drawn through the hair dryer unit as the anemometer only functioned in a range up to 80°C. These measurements are applicable as the only difference between using the unit hot and cold is the use of the heating element, no additional air is pulled in. Ten replicate measurements were taken from positions around the circular port. The mean was used in the calculation of volume flow rate of air into the boxes.

Replicate air speed measurements (metres/sec)				
0.38	0.34	0.34	0.35	0.36
0.36	0.35	0.37	0.37	0.35
max	0.38	min	0.34	
			mean	**0.36**

Table 64 Measurements of air speed exiting hair dryer unit (m/sec)

The diameter of the port was found to be 20mm=0.02m. Therefore the cross sectional area of the circle is:

$$\text{Area} = \pi r^2$$

$$\text{Area} = 3.141592654 \times (0.01^2)$$

$$\text{Area} = 0.000314159 m^2$$

The volume flow rate (m^3/sec)= Speed (m/sec) x cross sectional area (m^2)

$$\text{Flow rate} = 0.36 \times 0.000314159$$

$$\text{Flow rate} = 0.000113097 \ m^3/sec$$

This equates to 6.78l applied during a one minute application and 13.57l applied during a two minute application. Thus, the air applied via the hair dryer replaced 37.7% and 75.4% respectively of the box volume.

After removal of the resin sample tubes and sealing the access hatches the boxes were incubated at 10°C for the duration of the trial. The next sampling occasion was approximately two hours post-application. Headspace gas samples were removed from the top of the boxes via gas tight syringe and injected into the GC, however there was no detectable ethylene present. Tubes packed with absorbent resin as before were used to sample the box atmosphere. The ends of the tubes were connected to an air-sampling pump set at a rate of 50ml/min. The box air was sampled for 60 minutes giving a sample volume of 3l that passed over the resin bed. This procedure was carried out again at 24 hours post application. Upon removal of the tubes the access hatches were again sealed.

The above experiment was repeated with the application of cold air (17-18°C) for one or two minutes with a view to differentiating between the effect of forcing air through the storage facility alone and doing this with the added stress of high temperature. The only difference in sampling procedure with the cold treated replicates was that the sample time was reduced from 60 minutes to 50minutes at 50ml/min, thereby the total volume passed over the resin bed was 2.5l instead of 3l. This was merely a result of time restrictions.

The treatment variables are summarised in the table below.

Crop condition	Hot air	Cold air
good	1 min	1 min
good	2 mins	2 mins
poor	1 min	1 min
poor	2 mins	2 mins

Table 65 Summary of treatments and application carried out in Crop condition trial

The ethylene traps were desorbed and analysed as described in chapter 2.

The carbon dioxide traps were 50ml of 1M NaOH. When collected the bottles were sealed and reopened immediately prior to titration for quantitative analysis. The CO_2 reacts with the NaOH to form $NaHCO_3$. For titration, 15ml of 1M $BaCl_2$ was added to the trapping solution to precipitate out the $Ba(HCO_3)_2$. $Ba(HCO_3)_2$ is formed when the $BaCl_2$ reacts with the $NaHCO_3$, NaCl is also formed. The residual NaOH is titrated with 1M HCl. The

volume of HCl required dictates the amount of NaOH that was not reacted with CO_2 from respiration. This value can be used to extrapolate the amount of CO_2 that was trapped in the solution. The traps are considered to be relatively efficient as they are commonly used for respiration measurements when more advanced equipment is not available.

8.1.3.2 Results and Discussion

The results for ethylene concentration are expressed as nl/l (equal to ppb) representing the level present in box atmospheres. The volume used in the calculation is the amount of air drawn through the resin bed. In the case of the samples collected during application the volume used in the calculation is the total air forced into the box. Clearly the actual volume through the tube will be less than this because of the other sites of leakage (corners of boxes), but this is the theoretical maximum. Using this value generates the lowest possible value of concentration in store atmosphere and is accepted as an underestimate of the ethylene evolved by the potatoes. The samples collected at this point are more to show that ethylene is present rather than to quantify the release. The amount produced would have to be compared to the typical natural levels detected in work described earlier (Chapter 5) before a statement could be made about how soon the stress exerts itself on the ethylene synthesis cycle.

It is of interest to note that ethylene could be detected even when air was sampled under fluctuating conditions for only five or six minutes.

The table overleaf (*Table 68*) displays the ethylene concentrations found to be present in box atmospheres following treatment with cold or hot air at force. The mean value of the three replicates of each treatment is included in the table. Standard deviations are not given. The values are so low that any obvious errors were eliminated without the aide of statistics. The remaining results were acceptably reproducible for the experiment, within 0.6ppb and in most cases less.

Mean ethylene concentration ppb (nl/l) (to the nearest whole unit)				
Cold air		Good	Poor	Empty box
1 minute	At application	3	2	1
	2 hour post app	1	1	1
	24 hour post app	1	1	1
Cold air		Good	Poor	Empty box
2 minutes	At application	2	1	1
	2 hour post app	10	8	1
	24 hour post app	2	1	1
Hot air		Good	Poor	Empty box
1 minute	At application	0	0	0
	24 hour post app	3	3	0
Hot air		Good	Poor	Empty box
2 minutes	At application	0	0	0
	24 hour post app	1	1	0

Table 66 Mean ethylene concentration in the headspace of boxes receiving forced air treatment (ppb)

There was a low level of ethylene detected in all empty boxes on all occasions when cold air was applied at approximately 1ppb. This is considered to be the average background reading in these circumstances.

The only result that could be distinguished from the background value from crop treated with cold air for one minute was that taken at application, when the peak ethylene concentration was 2ppb for the crop in poor condition and 3ppb for the crop in good condition.

When the cold air treatment was increased to two minutes the stress effect was more pronounced overall. Although during application the crop did not respond as much as in the shorter treatment. Indeed the ethylene concentration in boxes of poor crop was not any different to that in the empty boxes, considered to be background level. Even the good crop peaked during application at only 2ppb. In spite of these relatively low levels during application the higher results from samples collected two hours post application (10 and 8ppb) show clearly that ethylene was synthesised and evolved at an elevated level in response to the stress exerted on the potatoes. This perceived stress appears to be transitory as by twenty-four hours after application levels had returned to values equalling those noted during application.

The crop in good condition responded consistantly to a greater degree when treated with cold air at force than the crop in poor condition. Perhaps when tubers are physiologically younger they are more ready and/or able to react defensively and increase ethylene production than older tubers.

With hot air applications the background level from empty boxes was found to be negligible on all sampling occasions. Bearing in mind the results are presented as whole units rounded up/down as applicable from the actual value to the nearest ppb, the zero figures represent a result below 0.5ppb. Given that these levels are extremely small a value of less than this could not be quantified with satisfactory confidence. Therefore it does not mean that no ethylene was detected, but rather that it was in extremely low levels. The use of whole units allows a quick comparison of the effect of crop condition and air treatments on ethylene evolution from tubers.

Hot air applications, both one and two minutes, did not generate increased ethylene synthesis in the same order as when cold air was blasted in for two minutes. Although an increase was noted after twenty-four hours in both instances, albeit small. The combined stress of forced air with high temperature was expected to be more detrimental in terms of ethylene production.

Crop in poor and good condition responded in exactly the same way when exposed to hot forced air. There seems to have been a delayed effect when compared to with results using cold air. Even without two-hour samples if the response were as immediate to hot air as it was to cold, higher values would have been noted during application. As this did not happen it is possible that the effect of forced air at relatively cool temperatures results in a faster triggering of defence mechanisms in the tubers than hot air that is forced into stores. Although further experimental work would be required to determine whether this is the case.

This is contrary to expectations of the behaviour of the potatoes exposed to elevated temperatures, presumed to be more stressful than the relatively cooler conditions. However it cannot be stated that this would remain the case if temperatures were up to 200°C upon entering potato stores. Temperatures in excess of 200°C at the end of the application pipe have been measured as part of this project (Chapter 4).

The detection of ethylene in the samples collected during application of air at force indicates that it caused the potatoes to produce ethylene. The boxes had been opened

immediately prior to application (to refresh CO_2 traps) and as such no ethylene exuded by the crop under normal conditions would have been remaining in the storage atmosphere when the lid was fitted. Hence, the ethylene detected in these samples was evolved during the course of application.

The respiration results below are expressed as mg CO_2 evolved/kg fresh weight/hour. The fresh weight in all samples was 5kg and the time of samples collection was twenty-four hours after application of air. Appropriate blanks from three empty boxes were included on each sampling occasion and the titration volume of the sample was subtracted from the mean blank titre value before calculation of CO_2 evolved.

Mean respiration rate (mg carbon dioxide evolved/kg fwt/hour)		
Cold air	**Good**	**Poor**
1 minute	**0.79**	**1.00**
2 minute	**0.77**	**0.92**
Hot air	**Good**	**Poor**
1 minute	**1.30**	**0.76**
2 minute	**1.28**	**0.72**

Table 67 Mean respiration rate of potatoes exposed to the stress of hot or cold air at force for one or two minutes

The crop in better condition respired less when treated with cold air than the more aged crop. Whether this would have been the case under normal conditions is unknown, but it is likely that its starting respiration rate would have been slightly higher because of its age. Generally once the crop has begun to respire at an accelerated rate because of external factors (i.e. previous exposure to CO_2 or C_2H_4) it will continue at an increased level although could possibly slow slightly when atmospheric conditions are more suitable. It is possible that in both sets of samples the increase is a result of the cold air treatments, but the crop in poor condition had a higher starting rate of oxygen consumption and CO_2 evolution.

There is only a slight difference in respiration rate between one and two minute applications, suggesting that for any difference in response to be noted, if at all it occurs, the difference in the time of the exposure to stress would have to have been much greater. On this time scale even though it has a substantial impact in 18l boxes it was not sufficient for the potatoes to perceive the longer application time as more stressful.

The results of the hot applications are the opposite of the reaction displayed when cold air is applied. Here the better quality potatoes respired more as a consequence of exposure to hot air at force. Again there was little difference between applications of one or two minutes. Both treatment durations were significantly detrimental to the potatoes.

8.1.3.3 Conclusions

It appears that the potatoes defensive response to stress depend not only on the nature of the stress but on the starting physical status of the crop.

Bearing in mind there is no exogenous source of ethylene applied and what is measured is purely that evolved by the tubers. It is probable that there is a temperature above which ethylene biosynthesis rates slow down. This seems sensible as normally there is a range of temperature in which a fruit or vegetable will behave normally, within reason, but at either extremes of this range physiological breakdown will be initiated.

An alternative reasoning for why tubers evolved more ethylene when treated with cold air and not hot could be that there was a rapid build up of carbon dioxide via increased respiration resulting from treatments. The supposed elevated carbon dioxide levels could be limiting the production of ethylene by restricting oxygen availability. Carbon dioxide is a known antagonist of the ethylene biosynthetic pathway.

9 Chapter Nine

9.1 Conclusions

9.1.1 Outcomes of this project

The main findings from this thesis based on the literature reviewed and the practical scientific and technical work conducted are as follows:

1. **That application of CIPC as a thermal fog under normal conditions was detrimental to the processing quality of potatoes in storage.**

The effect may be transitory and some recovery in fry colours observed over time, but it is unlikely that it will ever return to a level equal to pre-treatment. The effect was attributed to the ethylene present in the fogs as a by-product of hydrocarbon combustion and was not related to CIPC itself.

Carbon dioxide from the combustion process was not generated in high enough concentration to be the cause of this characteristic sugar spike and corresponding darker fry colours.

Depending on the storage facility (leakage, ventilation system and application procedure) ethylene could persist in store atmospheres for significant periods of time of at least twenty-four hours.

2. **Ethylene can have both positive and negative effects on potato tubers in long-term storage.**

Exposure of potatoes to ethylene caused reducing sugar concentration to increase and gave rise to a higher rate of respiration. When the exposure time was short and tubers were then returned to normal air conditions the ongoing effect from this exposure was stimulated sprout growth. This was found both in the data presented in this thesis and from literature (*Rylski et al, 1974, Alam et al, 1994*). When the exposure arose from CIPC application the stimulatory effect was obviously combated by the CIPC. In most cases the sprout control will be effective but it would be better to avoid the encouragement of dormancy break throughout long-term storage.

There appeared to be a short lag period between tuber exposure to exogenous ethylene and the elicited reaction, but a response was always noted. Methods that reduced or inhibited the effect of ethylene on sugars were either antagonistic e.g. elevated carbon dioxide or prevented ethylene binding to its receptors e.g. silver nitrate salts.

These chemicals also interfere with the natural biosynthetic pathway of ethylene in plant systems. This metabolic pathway is vital to the natural cycle of ethylene. It is an endogenous plant hormone that is evolved naturally by most if not all plant tissues and the rate of synthesis depends on stress levels perceived by the plant. The physiological status of the system will also influence the rate of production.

If potatoes are exposed to a consistent concentration of ethylene (approximately 1ppm and above) sprouting will be suppressed. The end of this effective concentration range is unclear but as there does not seem to be any benefit to sprout control of using levels greater than approximately 5ppm, this was not of grave concern. Although *Parkin & Schwobe, 1990*, stated that storage in 1000ppm of ethylene reduced the rate of conversion of sucrose to glucose and fructose.

At low levels of around 0.5ppm ethylene will have greater stimulatory effect on sprouting, but short bursts of exposure at any concentration, certainly within the range typical of fogging, will give rise to sprout growth.

3. **The negative effect of thermal fog application (ethylene) on fry colour can be reduced by simple changes to the routine procedure of thermal fogging.**

The effectiveness of the measures outlined below are likely to vary depending on cultivar and the physiological age of the potatoes.

The most practical and cost effective method of achieving this was to ventilate stores earlier after application. This shortened the exposure time of crop to contamination that caused reducing sugars to increase. It was shown that getting rid of ethylene by ventilating when the fog had cleared helped to maintain light fry colours. The useful fraction of the fog (particle size dependent) will have settled well within twenty-four hours and in most cases in less than eight (*BPC, summary leaflet, Dr J. Christiansen, personal communication*). Therefore loss of CIPC will be negligible by comparison with standard twenty-four hour ventilation. How soon fog settles would have to be determined on an individual store basis.

Technology transfer of the findings of this project through the BPC has meant that by and large in most commercial storage facilities this approach has been implemented. It is now uncommon to find a processing potato store where the ventilation interval is twenty-four hours after CIPC application.

Alternative cleaner fuels are an option for cutting down on the ethylene content of fogs. Methanol showed a vast improvement. Although LPG should in theory not have a negative effect on fry colour that equals the effect from petrol, this gas was not as successful as originally hoped. In its favour, LPG would be cheaper to run and considered more environmentally friendly.

LPG fogging systems are increasing in popularity in the UK, whether they will become the standard remains to be seen.

Tailoring the dose rate of CIPC applied was a controversial step because there are two strong opinions in the industry that hinge around this issue. Firstly that a reduced application rate would allow more frequent re-application at shorter intervals of twenty-eight days and hence provide better control. Conversely that the dose rate should be increased to extend the reapplication interval and aim towards reducing the total amount of CIPC applied. The argument here is that there will be less regular fogging events and as such crop will not be exposed to stressful conditions so frequently. Additionally it is a step toward lower residues because less will be applied. Both of these viewpoints have good reasoning, but evidence from this study indicates that it would be more beneficial to increase application rates if efficacy was not compromised.

The topics examined and discussed are intended to increase awareness and understanding not only of the thermal fogging application process but the behaviour and influence of ethylene in potato stores. The recommendations put forward will permit improvements in the fogging technique to be instigated and hopefully encourage further development of the system. This way good sprout control can be maintained throughout long-tem storage but not at the cost of a decline in processing quality

9.1.2 Update on the situation in the potato industry

In general all applicators are moving towards eliminating hydrocarbon fuels and developing heat exchangers. Currently gas heat exchangers are favoured in the UK because of the cost and logistical implications of using electric power.

In September 2003 the solid CIPC briquette was launched by Aceto (similar system has already been in use in the United States for some time). The block is heated and the molten CIPC is introduced to the hot air stream as with current fogging techniques. The benefits are that there is not solvent and therefore no additional supplementary chemicals going into store, and in the near future it will have a shorter application time. A shorter application time is claimed to be less stressful to the potatoes, but this has not been substantiated and cannot be until the machinery required is available.

Since the start of this research period Tecnazene use has been banned and all remaining stocks had to be used up or disposed of by February 2002. This means that CIPC is the only sprout suppressant chemical available for long-term storage of processing potatoes in the UK. Dimethylnaphthalene (DMN) is still in the process of registration. Di-isoproyl naphthalene (DIPN) has been recently introduced by Aceto, but it is still very much in its experimental years. Ethylene has been introduced as a new sprout suppressant and many companies are developing systems for generating and monitoring ethylene in storage facilities. As demonstrated in various experiments in this thesis the source of ethylene can be simply from a standard gas cylinder, a purpose built generator or even something as simple as apples. Providing a stable and uniform concentration in an appropriate range can be maintained sprouting should be effectively controlled while in store. The major drawback with this chemical is the effect on processing quality, which nullifies its use in the processing sector unless a method of avoiding the increased sugars can be established. This is currently under scrutiny and investigations of ethylene blocking chemicals intensify, principally with 1-methylcyclopropene (*Prange & DeLong, 2002-2003*).

Further research funded by the BPC is underway. These new projects are based on the results presented here and those of a more environmentally orientated project (*BPC, project 207, Park*). Pressure continues on store managers to meet the demands of large retailers, by cutting back on the amount of CIPC used without forgoing quality.

The areas of work now being investigated include:

To reduce total use of CIPC and hence residue levels;

At loading formulations (controlled release)

Potential vapour action

Methods to preserve quality while thermal fogging;

Catalytic converters

Heat exchangers

Contamination and decontamination studies concerning the environmental impact.

After a very long review process CIPC was granted Annex I listing recently. More so now than ever before, residues are a controversial subject, particularly with the step-up in monitoring and legislating environmental issues relating to its use, for example the changes to water policy that affect the potato industry (*Tompkins & Clayton, 2003*).

Evidently the impending MRL is a serious concern. Even with recent literature highlighting that 91-98% of total CIPC residues can be removed by peeling, the focus seems to go to the less optimistic finding that only 33-37% was removed by washing (*Lentza-Rizos & Balokas, 2001*). This is quite a reasonable proportion to be removed by gentle washing in tap water. This is why the suggestion of removing the time interval between the last CIPC application and sale to the public seems contradictory. *Hill & Reynolds, 2002*, surmised that residue concentrations could be expected to decline with time, irrespective of the pesticide, the crop or the prevailing conditions.

The Assured Produce protocol (*Dr H Duncan, personal communication*) introduced in 2003 was designed to improve and standardize the application procedure and requires more detailed store manager's records of events to ensure safe and effective use of CIPC. The Pro Potato website operated by Aceto is a very similar scheme of recommendations and contacts for advice. These are all produced in support of continued use of CIPC in UK and beyond. A further concern of consumers is product traceability. Global Potato News reported recently that Greenvale Ltd has introduced a system whereby an identification

number on each bag of potatoes bought in the supermarket could be used to trace on the internet, not only the source of crop but its history since leaving the farm.

Studies have continued into genetic modifications to develop tubers resistant to low temperature sweetening and with prolonged dormancy periods (*Sonnewald, 2001, Finlay et al 2003).* The integration of potatoes genetically modified in this way for general use is still a long off.

The principles of controlled atmosphere storage had been applied to packaging to help to preserve aesthetic and taste attributes of the potatoes while on display (*Hertog & Tijskens, 1998, Fonseca et al, 2002*). The concept is called modified atmosphere packaging and could conceivably be introduced for potatoes from ethylene storage to prevent the onset of vigorous sprouting upon leaving the store.

Public concerns and food safety issues have been raised regarding acrylamide levels in the diet and a possible cancer risk (*Tareke et al, 2002*). This has become an increasingly sensitive matter in the last two years culminating in a review by *Duncan & Hardie, 2003* on the acrylamide situation in the potato industry. In summary, acrylamide formation can be curbed by minimising the accumulation of reducing sugars (the limiting factor) in potato tubers. It is formed during processing when temperature conditions are high.

Bearing all of this in mind CIPC is still the main sprout suppressant relied upon for long-term storage and clearly efforts have been made by many in the industry to support it. Assuming the recommendations from this and other work (*BPC project 207*) on improving the application and efficiency of CIPC are implemented, it is reasonable to speculate that use of CIPC at least in the UK can be continued well into the future.

10 References

Abdel-Rahman A.A. (1998): On the emissions from internal combustion engines: a review, *International Journal of Energy Research, 22, 483-513*

Abeles F.B. (1973): Ethylene in Plant Biology, *Whistler and Wolfrom, Academic Press, London*

Abeles F.B. (1992): Ethylene in Plant Biology, *Whistler and Wolfrom, Academic Press, London, 2nd edition*

Agatsuma M. and T. Tamura (1973): Ethylene evolution in apple tissue and seed at various stages of maturity, *Journal of the Faculty of Agriculture, Hokkaido University, 57, 3*

Agblor A. and M.G. Scanlon (2002): Effect of storage period, cultivar and two growing locations on the processing quality of french fried potatoes, *American Journal of Potato Research, 79, 167-172*

Alam S.M.M, D.P. Murr and L. Kristof (1994): The effect of ethylene and inhibitors of protein and nucleic acid syntheses on dormancy break and subsequent sprout growth, *Potato Research, 37, 25-33*

Allen C.A.W. and K.C. Watts (2000): Comparative analysis of the atomization characteristics of fifteen biodiesel fuel types, *Transactions of the ASAE, 43, 207-211*

Alltech Associates Inc (1998): Air quality monitoring: Part 1 of 4, An introduction to why and how, *Alltech offices*

Asfar K.R. and H. Hamed (1998): Combustion of fuel blends, *Energy Conservation and Management, 39, 1081-1093*

Barichello V., R.Y. Yada and R.H.Coffin (1991): Starch properties of various potato (Solanum tuberosum L) cultivars susceptible and resistant to low-temperature sweetening, *Journal of the Science of Food and Agriculture, 56, 385-397*

Beechman H. H., M.F. Dull (1951): Amino sugar interactions, *Food Research, chapter 16, 439*

Bertsch W., A. Zlatkis, H.M. Liebich and H.J. Schneider (1974): Concentration and analysis of organic volatiles in skylab 4, *Journal of Chromatography, 99, 673-687*

Beveridge J.L., J. Dalziel and H.J Duncan (1983): headspace analysis of laboratory samples of potato tubers treated with 1,4-dimethylnaphthalene, carvone, pulegone and citral, *Journal of the Science of Food and Agriculture, 34 (2), 164-168*

Black M.S., W.R. Rheg, R.E. Sievers and J.J. Brooks (1977): Gas chromatographic technique for compound class analysis of jet engine exhaust, *Journal of Chromatography, 142, 809-822*

Bleeker B.B. and H. Kende (2000): Ethylene: a gaseous signal molecule in plants, *Annual Review of Cell Division and Biology, 16, 1-8*

Blenkinsop R.W., L.J. Copp, R.Y. Yada and A.G. Marangoni (2002): Effect of chlorpropham (CIPC) on carbohydrate metabolism of potato tubers during storage, *Food Research International, 35, 651-655*

Boyd I.M.G (1988): The effect of sprout suppressant chemicals on the sugar content of potato tubers, *Glasgow University PhD, chapter six*

Breech D.L., M. Workman and M.D Harrison (1973): The influence of storage factors on endogenous ethylene production by potato tubers, *American Potato Journal, 50, 145-150*

Brierley E.R., P.L.R. Bonner and A.H. Cobb (1996): Factors influencing the free amino acid content of potato (Solanum tuberosum L.) tubers during prolonged storage, *Journal of the Science of Food and Agriculture, 70, 4, 515-525*

Brierley E.R., P.L.R. Bonner and A.H. Cobb (1997): Aspects of amino acid metabolism in stored potato tubers (cv. Pentland Dell), *Plant science, 127, 17-24*

Briddon A. and A. Jina (1999): The effects of carbon dioxide on the processing quality of stored potatoes, *BPC Project summary leaflet*

Bunch J.E, E.D. Pellizzari (1979): Evaluation of chromatographic sorbents used in air pollution studies, *Journal of Chromatography, 186, 811-829*

Burfoot D., D.L.O. Smith, M.C. Butler-Ellis and W. Day (1996): Modelling the distribution of isopropyl N-(3-chlorophenyl) carbamate [CIPC] in box potato stores, *Potato Research, 39, 241-251*

Burg P. and E.A. Burg (1965): Molecular requirements for the biological activity of ethylene, *Plant Physiology, 42, 144-152*

Burton W.G (1952): Physiological effects of the volatile products of respiring potatoes, *Nature, 169, 117*

Burton W.G. and D.F. Meigh (1971): The production of growth-suppressing volatile substances by stored potato tubers, *Potato Research, 14, 96-101*

Burton W.G (1989): The Potato, *Longman Publishers, 424*

Burton W.G.: Methods of assessments for potatoes and potato products, *European Association for potato research*

Canizares-Macias P., L. Hernandez-Garciadiego and H. Gomez-Ruiz (2001): An automated flow injection analysis procedure for the determination of reducing sugars by DNSA method, *Journal of Food Science, 66, 407-411*

Cardini C.E., L.F. Leloir and J. Chiriboga (1955): *Journal of Biological Chemistry, 214, 149*

Cargill B.F., D.R. Heldman and C.L. Bedford (1971): Influence of environmental storage conditions on potato chip quality, *Potato Chipper, October*

Chevery J.L., M.O. Sy, J. Pouliqueen and P. Marcellin (1988): Regulation by carbon dioxide of 1-aminocycloproane-1-carboxylic acid conversion to ethylene in climateric fruits, *Physiologia Plantarum, 72, 535-540*

Coleman W.K. (1987): Dormancy release in potato tubers: a review, *American Potato Journal, 64, 57-68*

Coleman W.K., G.C.C. Tai, S. Clayton, M. Howie and A. Pereria (1993): A portable monitor for the rapid assessment of processing quality of stored potato tubers, *American Potato Journal, 70, 909-923*

Coleman W.K. and J. McInerney (1997): Enhanced dormancy release and emergence from potato tubers after exposure to a controlled atmosphere, *American Potato Journal, 74, 173-182*

Coleman W.K. (1998): Carbon Dioxide, oxygen and ethylene effects on potato tuber dormancy release and sprout growth, *Annals of Botany, 82, 21-27*

Coleman W.K. and G.C.C. Tai (1999): Comparative performance of three test methods for determining chipping quality in tubers of potato (Solanum tuberosum L.), *Canadian Journal of Plant Science, 79, 4, 625-632*

Coleman W.K. (2000): Physiological ageing of potato tubers: a review, *Annals of Applied Biology, 137, 189-199*

Copp L.J., R.W. Blenkinsop, R.Y. Yada and A.G. Marangoni (2000): The relationship between respiration and chip colour during long-term storage of potato tubers, *American Journal of Potato Research, 77, 279-287*

Corsini D., G. Stallknecht and W. Sparks (1979): Changes in chlorpropham residues in stored potatoes, *American Potato Journal, 56, 43-50*

Coxon D.T. and A.A.E. Filmer (1985): The fate and distribution of chlorpropham when applied to stored potatoes as a sprout suppressant, *Pesticide Science, 16, 355-363*

Craven M.A., J.W. Gardner and P.N. Bartlett (1996): Electronic noses - development and future prospects, *Trends in Analytical Chemistry, 15, 9, 486-493*

Duncan H.J., I.M.G. Boyd and J.W. Muir (1992): Methods for controlling sprouting in potatoes, *Aspects of Applied Biology, 33, 189-196*

Duncan H.J., A.C.C. Cunnington, A. Briddon, A. Jina, P.C.H. Miller and A.G. Lane (2001): Optimisation of CIPC application and distribution in stored potatoes, *BPC Project report number 2001/6*

Duncan H.J. and S. Hardie (2003): Literature review on the status of amino acids and soluble sugars on the formation of acrylamide in processed potatoes, *FNK Potato Processors Research Group*

Duplessis P.M., A.G. Marangoni and R.Y. Yada (1996): A mechanism for low temperature induced sugar accumulation in stored potato tubers: the potential role of the alternative pathway and invertase, *American Potato Journal, 73, 483-494*

Emond J.P., K.V. Chau, J.K. Brecht and M.O. Ngadi (1998): Mathematical modelling of gas concentration profiles in modified atmosphere bulk packages, *Transactions of the ASAE, 41, 4, 1075-1082*

European Association of Potato Processing Industry (2002): Minutes of CIPC working group meeting, *Munich February 2000*

Espen L., S. Morgutti, A. Abruzzese, N. Negrini, A. Rivetta, M.M. Quattrini, M.Cocucci and S.M. Cocucci (1999): Changes in the potato (Solanum tuberosom L.) tuber at the onset of dormancy and during storage at 23 degrees C and 3 degrees C. I. Biochemical and physiological parameters, *Potato Research, 42, 189-201*

Evans J. and N.P. Batty (1994): Ethylene precursors and antagonists increase embryogenesis of Hordeum vulgare L. anther culture, *Plant Cell Reports, 13, 676-678*

Ewing E.E., J.W. Layer, J.C. Bohn and D.J. Lisk (1968): Effects of chemical sprout inhibitors and storage conditions on internal sprouting in potatoes, *American Potato Journal, 45, 56-71*

Fan X and J.P. Mattheis (2000): Yellowing of broccoli in storage is reduced by 1-methylcyclopropene, *Horticultural Science, 35, 5, 885-887*

Fedtke C (1982): Biochemistry and physiology of herbicide action, *Springer-Verlag NY Inc.*

Filmer A.A.E. and D.G. Land (1978): The accumulation of volatile substances in a large modern potato store, *Journal of the Science of Food and Agriculture, 29, 219-224*

Filmer A.A.E. and M.J.C. Rhodes (1985): Investigation of the sprout-growth-inhibitory compounds in the volatile fraction of potato tubers, *Potato Research, 28, 361-377*

Fick R.J. and R.C. Brook (1999): Threshold sugar concentrations in Snowden potatoes during storage, *American Journal of Potato Research, 76, 357-362*

Finlay M., B. Dale and J.E. Bradshaw (2003): Progress in improving processing attributes in potatoes, *Trends in Food Science, 8, 7, 310-312*

Fonseca S.C., F.A.R. Oliveira and J.K. Brecht (2002): Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review, *Journal of Food Engineering, 52, 9-119*

Freidrich T. (2000): Pesticide application and the environment: the difficulty of making the right descion, *Phytoparasitica, 28, 3, 199-201*

Gamas E.D., L. Diaz, R. Rodriguez, E. Lopez-Salinas, I. Schifter and L. Ontiveros (1999): Exhaust emissions from gasoline- and LPG- Powered vehicles operating at the altitude of Mexico City, *Journal of Air and Waste Management Association, 49, 1179-1189*

Glennie J (2001): *Glasgow University project report*

Habib A.T. and H.D. Brown (1956): Factors influencing the colour of potato chips, *Food Technology, July, 332-336*

Hajslova J. and J. Davidek (1986): Sprout inhibitors IPC and CIPC in treated potatoes, *Die Nahrung, 30, 1, 75-79*

Hassan-Amrani M.E., R.M. Dowdeswell, P.A. Payne and K.C. Persaud (1997): An intelligent gas sensing system, *Sensors and Actuators B, 44, 512-516*

Hay R.K.M. and J. Hampson (1991): Sprout and stem development from potato tubers of differing physiological age: the role of apical dominance, *Field Crops Research, 27, 1-16*

Herregods M. (1995): Preservation of the quality and nutritional value of fruit and vegetables by ca storage, *Acta Horticulturae, 379, 321-329*

Herrman T.J., S.L. Love, B. Shafii and R.B. Dwelle (1996): Chipping performance of three processing potato cultivars during long-term storage at two temperature regimes, *American Potato Journal, 73, 9, 411-425*

Hertog M.L.A.T.M., B. Putz and L.M.M. Tijskens (1997): The effect of harvest time on the accumulation of reducing sugars during storage of potato (Solanum tuberosum L.) tubers: experimental data described, using a physiological based, mathematical model, *Potato Research, 40, 1, 69-78*

Hertog M.L.A.T.M. and L.M.M. Tijskens (1998): Modelling modified atmosphere packing of perishable produce: keeping quality at dynamic conditions, *Acta Alimentaria, 27, 1, 53-62*

Hill T.A (1980): Endogenous plant growth substances, *Edward Arnold Ltd*

Hill A.R.C. and S.L. Reynolds (2002): Unit-to-unit variability of pesticide residues in fruit and vegetables, *Food Additives and Contaminants, 19, 8, 733-747*

Hironaka K., K. Ishibashi and K. Hakamada (2001): Effect of static loading on sugar contents and activities of invertase, UDP-glucose pyrophosphorylase and sucrose 6-phosphate synthase in potatoes during storage, *Potato Research, 44, 33-39*

Huxham M. and C.L.P. Thomas (2000): Sampling procedures for intrinsically valid volatile organic compound measurements, *Analyst, 125, 825-832*

Jankowski K.M., K.L. Parkin and J.H. VonElbe (1997): Nonuniform browning or "mottling" in French fry products associated with a heterogeneous distribution of reducing sugars, *Journal of Food Processing and Preservation, 21, 1, 33-54*

Jarvis M.C., J. Dalziel and H.J. Duncan (1974): Variations in free sugars and lipids in different potato varieties during low-temperature storage, *The Journal of the Science of Food and Agriculture, 25, 1405-1409*

Jeong J.C., R.K. Prange and B.J. Daniels-Lake (2002): Long-term exposure to ethylene affects polyamine levels and sprout development in 'Russet Burbank' and 'Shepody' potatoes, *Journal of the American Society of Horticultural Science, 127, 1, 122-126*

Jiang Y., D.C. Joyce and A.J. Macnish (1999): Responses of banana fruit to treatment with 1-methylcyclopropene, *Plant Growth Regulation, 28, 77-82*

John P. (1997): Ethylene biosynthesis: the role of 1-aminocyclopropane-1-carboxylate(ACC) oxidase, and its possible evolutionary origin, *Physiologia Plantarium, 100, 583-592*

Johnson P.R. and J.R. Ecker (1998): The ethylene gas signal transduction pathway: a molecular perspective, *Annual Reviews of Genetics, 32, 227-254*

Kader A.A. (1986): Biochemical and physiological basis for effects of controlled and modified atmospheres on fruits and vegetables, *Food Technology, 40, 5, 99-101*

Kalt W., R.K. Prange and B.J. Daniels-Lake (1999): Alternative compounds for the maintenance of processing quality of stored potatoes, *Journal of Food Processing Preservation, 23, 71-81*

Khanbari O.S. and A.K. Thompson (1996): Effect of controlled atmosphere, temperature and cultivar on sprouting and processing quality of stored potatoes, *Potato Research, 30, 523-531*

King G.A. and E.M. O'Donoghue (1995): Unravelling senescence: new opportunities for delaying the inevitable in harvested fruit and vegetables, *Trends in Food Science and Technology, 6, 385-389*

Kirkwood R.C (1991): Target sites for herbicide action, *Plenum*

Koivistoinen P. and A. Kavinpaa (1965): Stability of Isopropyl N-phenylcarbamate and Isopropyl N-(3-chlorophenyl)carbamate residues on fruit after harvest, *Postharvest Preservatives, 13 (5), 459-468*

Konrad S. and A. Volz-Thomas (2000): Characterization of a commercial gas chromatography-flame ionization detection system for the *in situ* determination of Carbon5-Carbon10 hydrocarbons in ambient air, *Journal of Chromatography A, 878, 215-234*

Kushalappa A.C., L.H. Lui, C.R. Chen and B. Lee (2002): Volatile fingerprinting (SPME-GC-FID) to detect and discriminate diseases of potato tubers, *Plant Disease, 86, 131-137*

Laza M., M.G. Scanlon and G. Mazza (2001): The effect of tuber pre-heating temperature and storage time on the mechanical properties of potatoes, *Food Research International, 34, 659-667*

Lentza-Rizos C. and A. Balokas (2001): Residue levels of chlorpropham i individual tubers and composite samples of post harvest treated potatoes, *Journal of Agricultural and Food Chemistry, 49, 710-714*

Leszkowiat M.J., V. Barichello, R.Y. Yada, R.H. Coffin, E.C. Lougheed and D.W. Stanley (1990): Contribution of sucrose to nonenzymatic browning in potato chips, *Journal of Food Science, 55, 1, 281-284*

Lougheed E.C. (1987): Interactions of oxygen, carbon dioxide, temperature, and ethylene that may induce injuries in vegetables, *Horticultural Science, 22, 5*

Lyew D., Y. Gariepy, C. Ratti, G.S.V. Raghavan, and A.C. Kushalappa (1999): An apparatus to sample volatiles in a commercial potato storage facility, *Applied Engineering in Agriculture, 15, 3, 243-247*

Maga J.A. (1994): Potato flavour, *Food Reviews International, 10, 1-48*

Mann J.D., J.P. Lammerink, G.D. Coles (1991): Predicting potato crisp darkening: two methods for analysis of glucose, *New Zealand journal of Crop and Horticultural Science, 19, 199-201*

Marangoni A.G., P.M. Duplessis, R.W. Lencki and R.Y. Yada (1996): Low-temperature stress induces transient oscillations in sucrose metabolism in solanum tuberosum, *Biophysical Chemistry, 61, 177-184*

Marangoni A.G., P.M. Duplessis and R.Y. Yada (1997): Kinetic model for carbon partitioning in Solanum tuberosum tubers stored at 2 degrees C and the mechanism for low temperature stress-induced accumulation of reducing sugars, *Biophysical Chemistry, 65, 2-3, 211-220*

Mares D. J., J.R. Sowokinos and J.S. Hawker (1985): Carbohydrate metabolism in developing potato tubers, *Potato Physiology, 279-327, Academic Press, New York*

Marquez G. and M.C. Anon (1986): Influence of reducing sugars and amino acids in the colour development of fried potatoes, *Journal of Food Science, 51, 1, 157-160*

Mathooko F.M. (1996): Regulation of ethylene biosynthesis in higher plants by carbon dioxide, *Post harvest Biology and Technology, 7, 1-26*

Mathooko F.M. (1996): Regulation of respiratory metabolism in fruits and vegetables by carbon dioxide, *Post harvest Biology and Technology, 9, 247-264*

Mazza G. (1983): Correlations between quality parameters of potatoes during growth and long-term storage, *American Potato Journal, 60, 145-159*

Mazza G. (1983): A comparison of methods of analysis for sugars in potato tubers, *Canadian Institute of Food Science and Technology Journal, 16, 4, 234-236*

Mazza G. and A.J. Siemens (1990): Carbon dioxide concentration in commercial potato storages and its effect on quality of tubers for processing, *American Potato Journal, 67, 121-132*

Meigh D.F. (1969): Suppression of sprouting in stored potatoes by volatile organic compounds, *Journal of the Science of Food and Agriculture, 20, 159-164*

Melton S.L., M.K. Trigiano, M.P.Penfield and R. Yang (1993): Potato chips fried in canola and/or cottonseed oil maintain high quality, *Journal of Food Science, 58, 5, 1079-1083*

Misener G.C., W.A. Gerber, G.C.C. Tai and E.J. Embleton (1996): Measurement of glucose concentrations of potato extract using a blood glucose test strip, *Canadian Agricultural Engineering, 38, 1, 59-62*

Moore T.M (1979): Biochemical and physiology of plant hormones, *Springer-Verlag NY Inc.*

M'Glasson W.B. (1969): Ethylene production by slices of green banana fruit and potato tuber tissue during the development of induced respiration, *Australian Journal of Biological Science, 22, 489*

Nagami H. (1997): Residues of maleic hydrazide and chlorpropham in potato chips, *Bulletin of Environmental Contamination and Toxicology, 58, 764-768*

Nagayama T. and K. Kikugawa (1992): Influence of frying and baking on chlorpham residue, *Japanese Journal of Toxicology and environmental Health, 38, 1, 78-83*

Nowak J. (1977): Biochemical changes in stored potato tubers with different rest periods. I. Influence of the storage temperature and isopropyl phenylcarbamates (IPC and CIPC) on protein changes, *ZeitschrifePflanzenphysiology, 81, 113-124*

NuChem: Materials safety data sheet

Okazawa Y. (1974): A relation between ethylene evolution and sprouting of potato tuber, *Journal of the Faculty of Agriculture, Hokkaido University, 57, 443-454*

Orr P.H., J.L. Varns and K.G. Janardan (1994): Predicting the response of potatoes to post-storage handling stress, *Transactions of the ASAE, 37, 6, 1907-1911*

Oruna-Concha M.J., S.C. Duckham and J.M. Ames (2001): Comparison of volatile compounds isolated from the skin and flesh of four potato cultivars after baking, *Journal of Agricultural and Food Chemistry, 49, 2414-2421*

Ozdogan S., S. Uygur and N. Egrican (1997): Formation and dispersion of toxic combustion by-products from small scale combustion systems, *Energy, 22, 7, 681-692*

Ozkan H.E. (1999): Recommendations for pesticide applicator training in USA based on licensing and training procedures in Western Europe, *Applied engineering in Agriculture, 15, 1, 15-20*

Parkin C.S., A.J. Gilbert, E.S.E. Southcombe and C Marshall (1994): British crop protection council scheme for the classification of pesticide application equipment by hazard, *Crop Protection, 13, 4, 281-285*

Parkin K.L. and M.A. Schwobe (1990): Effects of low temperature and modified atmosphere on sugar accumulation and chip colour in potatoes (Solanum tuberosum), *Journal of Food Science, 55, 5, 1341-1344*

Peshin A. (2000): Influence of storage temperature and reconditioning on the biochemical composition of potato tubers, *Journal of Food Science and Technology, 37, 2, 126-129*

Prange R.K., W. Kalt, B. Daniels-Lake, C. Liew, R. Page, J. Walsh, P. Dean and R. Coffin (1998): Using ethylene as a sprout control agent in stored 'Russet Burbank' potatoes, *Journal of American Society of Horticultural Science, 123, 463-469*

Prange R.K and J.M DeLong (2002-2003): 1-Methylcyclopropene: The 'magic bullet' for horticultural products?, *Unknown source*

Pritchard M.K. and L.R. Adam (1994): Relationships between fry colour and sugar concentration in stored Russet Burbank and Shepody potatoes, *American Potato Journal, 71, 59-68*

Pritchard M.K. and M.G. Scanlon (1997): Mapping dry matter and sugars in potato tubers for prediction of whole tuber process quality, *Canadian Journal of Plant Science, 77, 3, 461-467*

Ratcliffe N.M. (1999): An early warning system for disease detection in stored potatoes, *BPC project report, 807/172*

Reust W., P. Gugerli (1984): Oxygen and carbon dioxide treatment to break potato tuber dormancy for reliable detection of potato virus Y (PVY) by enzyme-linked immunosorbent assay (ELISA), *Potato Research, 27, 435-439*

Rodriguez-Saona L.E. and R.E. Wrolstad (1997): Influence of potato composition on chip color quality, *American Potato Journal, 74, 2, 87-106*

Rodriguez-Saona L.E., R.E. Wrolstad and C. Pereira (1997): Modelling the contribution of sugars, ascorbic acid and amino acids to non-enzymatic browning of potato chips, *Journal of Food Science, 62, 5, 1001-1010*

Roe M.A. and R.M. Faulks (1991): Colour development in a model system during frying - role of individual amino-acids and sugars, *Journal of Food Science, 56, 6, 1711-1713*

Roe J.H. (1934): *Journal of Biological Chemistry, 107, 15*

Rossell J.B. (1998): Industrial frying process, *Grasas y Acietes, 49, 282-295*

Rylski I., L. Rappaport and H.K. Pratt (1974): Dual effects of ethylene on potato dormancy and sprout growth, *Plant Physiology, 53, 658-662*

Sahin S. (2000): Effects of frying parameters on the colour development of fried potatoes, *European Food Research and Technology, 211, 165-168*

Saltveit M.E. (1999): Effect of ethylene on the quality of fresh fruit and vegetables, *Post harvest Biology and Technology, 15, 279-292*

Schutz S., B. Weissbecker and H.E. Hummel (1996): Biosensor for volatiles released by damaged plants, *Biosensors and Bioelectronics, 11, 4, 427-433*

Schutz S., B. Weissbecker, U.T. Koch and H.E. Hummel (1999): Detection of volatiles released by diseased potato tubers using a biosensor on the intact insect antennae, *Biosensors and Bioelectronics, 14, 221-228*

Shah N (1997): Ethylene and CIPC, *Glasgow University project report*

Sisler E.C. and C. Wood (1988): Interaction of ethylene and carbon dioxide, *Physiologia Plantarum, 73, 440-444*

Sisler E.C. and M. Serek (1999): Compounds controlling the ethylene receptor, *Botanical Bulletin of Academia Sinica, 40, 1-7*

Sinclair S.W. (1998): The production and effect of ethylene resulting from thermal fogging operations in a potato store, *Glasgow University project report*

Sloof M., L.M.M. Tijskens and E.C. Wilkinson (1996): Concepts for modelling the quality of perishable products, *Trends in Food Science and Technology, 7, 165-171*

Smalle J. and D. Van Der Straeten (1997): Ethylene and vegetative development, *Physiologia Plantarum, 100, 593-605*

Sonnewald U. (2001): Control of potato tuber sprouting, *Trends in Plant Science, 6, 8, 333-335*

Sowokinos J.R. (1978): Relationship of harvest sucrose content to processing maturity and storage life of potatoes, *American Potato Journal, 55, 333-344*

Suttle J.C (1996): Plant Dormancy, *CAB International, p133*

Tareke E., P. Rydberg, P. Karlsson, S Eriksson and M Tornqvist (2002): Analysis of acrylamide, a carcinogen formed in heated foodstuffs, *Journal of Agriculture and Food Chemistry, 50, 17, 4998-5006*

Tiainen T. (1992): The role of ethylene and reducing agents on anther culture response of tetraploid potato (Solanum tuberosum L.), *Plant Cell Reports, 10, 604-607*

Timm H., D.L. Hughes and M.L. Weaver (1986): Effect of exposure time of ethylene on potato sprout development, *American Potato Journal, 63, 655-664*

Timm H. and G.H. Timm (1985): Ethylene production response of unsprouted and sprouted seed potato pieces to temperature elevation, *American Potato Journal, 62, 444*

Tompkins J and R. Clayton (2003): Changes to water policy and their effect on the potato industry, *British Potato Council publication*

Tonneijck A.E.G., B.P. Jansen and C. Bakker (2000): Assessing the effects of atmospheric ethylene on epinasty and tuber yield of potato (*Solanum Tuberosum L.*) near polyethylene manufacturing plants, *Environmental monitoring and assessment, 60, 57-69*

Uppal D.S. (1999): Effect of storage environments on chip colour and sugar levels in tubers of potato cultivars, *Journal of Food Science and Technology, 36, 6, 545-547*

Vacha G.A. and R.B. Harvey: The use of ethylene propylene and similar compounds in breaking the rest period of tubers, bulbs, cuttings and seeds, *Plant Physiology, 187-193*

Varnes J.L. (1979): Defining potato stress by metabolic volatiles, *Chipper/Snacker, September, 5*

Varnes J.L. and M.T. Glynn (1979): Detection of disease in stored potatoes by volatile monitoring, *American Potato Journal, 56, 185-197*

Vaughn S.F. and G.F.Spencer (1993): Naturally occurring aromatic compounds inhibit potato tuber sprouting, *American Potato Journal, 70, 527-533*

Warner K., P. Orr, L. Parrot and M. Glynn (1994): Effects of frying oil composition on potato chip stability, *Journal of American oil Chemistry, 71, 10, 1117-1121*

Wang L. and M.K. Pritchard (1997): Ethylene contamination during CIPC application increases respiration and sugars of stored potatoes, *American Potato Journal, 74, 475*

Waterer D.R. and M.K. Pritchard (1984): Monitoring of volatiles: A technique for detection of soft rot (Erwinia carotovora) in potato tubers, *Canadian Journal of Plant Pathology, 6, 165-171*

Watkins C.B., J.F. Nock and B.D. Whitaker (2000): Responses of early, mid and late season apple cultivars to post harvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage solutions, *Post harvest Biology and Technology, 19, 17-32*

Weissbecker B., S. Schutz, A. Klein and H.E. Hummel (1997): Analysis of volatiles emitted by potato plants by means of a Colorado beetle electroantennographic detector, *Talanta, 44, 2217-2224*

Wiltshire J.J.J., A.H. Cobb (1996): A review of the physiology of potato tuber dormancy, *Annals of Applied Biology, 129, 553-569*

Whistler R.L., M.L. Wolfrom (1962): Methods in carbohydrate chemistry (Vol.1), *Academic Press*

Woolfe J.A (1987): The potato in the human diet, *Cambridge*

Wunch S., and Schaller (1972): Interactions of sugars and amino acids in the development of colour in potato crisps, *Potato Research, 12, 12-23*

Yang J., J.R. Powers, T.D. Boylston and K.M. Weller (1999): Sugars and free amino acids in stored Russet Burbank potatoes treated with CIPC and alternative sprout inhibitors, *Journal of Food Science, 64, 4, 592-596*

Zoccolillo L., D. Babi and M. Felli (2000): Evaluation of polycyclic aromatic hydrocarbons in gasoline by HPLC and GC-MS, *Chromatographia, 52, 5/6, 373-376*