CRANFIELD UNIVERSITY

SULAIMAN SOWE

THE EFFECTS OF CONTROLLED ATMOSPHERE AND ETHYLENE ON THE POSTHARVEST QUALITY OF SWEET POTATO DURING STORAGE

SCHOOL OF WATER, ENERGY AND ENVIRONMENT
Postharvest Technology

PhD
Academic Year: 2014 - 2018

Supervisor: Professor Leon .A. Terry
Associate Supervisor: Professor Andrew Thompson
April, 2018

CRANFIELD UNIVERSITY

SCHOOL OF WATER, ENERGY AND ENVIRONMENT
Postharvest Technology

PhD

Academic Year: 2014 - 2018

SULAIMAN SOWE

The Effects of Controlled Atmosphere and Ethylene on the Postharvest Quality of Sweet Potato during Storage

Supervisor: Professor Leon .A. Terry
Associate Supervisor: Professor Andrew Thompson
April, 2018

This thesis is submitted in partial fulfilment of the requirements for the degree of PhD

ABSTRACT

Sweet potato (*Ipomoea batatas* Lam) is the third most important food commodity in Sierra Leone and global demand for the commodity is growing. To meet this demand requires the use of effective storage methods due to the perishability (shrivelling, sprouting and disease) of the root after harvest. This study therefore investigated the efficacy of controlled atmosphere (CA) and ethylene on the physiological, biochemical, mycological and molecular dynamics of sweet potato during storage. This was done by storing sweet potato samples (cultivar: 06-52; known as Belle Vue in the USA) at 20˚C as follows: CA (5 kPa CO_2 and 8 kPa O_2), air (0.003 kPa CO_2 and 21 kPa O_2), CA supplemented with 0.001 kPa ethylene and continuous exogenous ethylene (0.001 kPa) for a maximum of 12 weeks. A transition phase was established at six weeks of storage which involved swapping of treatments from CA with and without supplemented ethylene to air storage and vice versa (Experiment 2) and that from ethylene to air storage and vice versa (Experiment 4).

Results showed that in as much as the ethylene supplementation was successful in supressing sprouting, the contents of phenolics and sugars increased as well as weight loss and respiration rates, which undermine its positive aspects. Storage in CA reduced weight loss and respiration rates but did suppress sprouting as well. The complete inhibition of sprouting during storage could also be attributed to decreased biosynthesis of the cytokinin, trans-zeatine riboside (trans-ZR) in the sweet potato due to ethylene supplementation. Also ethylene induced rise in phenolics corresponded with an increased relative expression of ethylene response factors (ERF) in the proximal section unlike the reducing sugars. The ERF gene was more expressed towards the end of storage whilst ethylene insensitive 2 (EIN2) was more expressed at the beginning which suggested that EIN2 was actively repressed during storage particularly under CA treatment.

Furthermore, continuous CA storage was effective in mitigating disease development on the sweet potato as well as in controlling *Penicillium* development but not that of *Fusarium*. Major diseases identified on the sweet potato were *Fusarium* surface rot and *Rhizopus* soft rot predominantly at the proximal and distal sections mainly during storage under ethylene supplemented CA. The mycotoxin, aflatoxin G1 was the most

predominant potentially produced aflatoxin on the sweet potato and continuous CA was very effective in inhibiting the potential contamination of all the studied aflatoxins: aflatoxin B1 (AFB1), aflatoxin B2 (AFB2) and aflatoxin G1 (AFG1) during storage.

Keywords: weight loss; respiration rates; disease; sprouting; non-structural carbohydrates; phenolics; aflatoxin, relative expression; phytohormones; biosynthesis

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to the Commonwealth scholarship commission, United Kingdom, for their financial and moral support in providing me the opportunity to have this great experience.

Special thanks also go to my supervisor Professor Leon Terry for his professional guidance and also to Dr. Sandra Landahl and Dr. Roberta Tosetti for their relentless support in seeing me through to the end. My thanks also go to members of the Plant Science and Applied Mycology groups for their technical assistance and guidance throughout my PhD program. I am also indebted to Pat Bellamy for her support in data analysis.

I also wish to also acknowledge the contributions of Barfoots of Botley Ltd., West Sussex, UK, for the supply of sweet potatoes used to conduct all my experiments and for also providing the accompanied relevant information needed, particularly Harry Wilder and Adam Stagg.

My sincere thanks and appreciation also go to my parents Mr and Mrs Ibrahim Sowe for their unwavering support and also my wife, Mrs Mariama Sowe, for her moral support, patience and courage during the period of my PhD program.

My gratitude goes to Zoe Payne for her administrative support in making sure that things are done on time.

TABLE OF CONTENTS

LIST OF FIGURES

APPENDICES

LIST OF TABLES

APPENDICES

LIST OF ABBREVIATIONS

CA	controlled atmosphere
CAE	controlled atmosphere supplemented with ethylene
CFU	colony forming unit
DFID	Department for International Development
kPa	kilo pascals
kg	kilogram
L	litre
1-MCP	1-methylcyclopropene
mg	milligram
mL	millilitre
ng	nanogram
PSL	Plant Science Laboratory
µL	microliter
et al.	and others
PDA	Potato Dextrose Agar
DG-18	Dichloran Dextrose Agar-18
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
MEA	Malt Extract Agar
ERF	Ethylene response factors
EIN2	Ethylene insensitive 2
EIN3	Ethylene insensitive 3
ACC	1-aminocyclopropane-1-carboxylic acid
ACO	S-adenosyl methionine oxidase
ACS	S-adenosyl methionine synthase
YES	Yeast Extract Sucrose Agar
CO_2	carbon dioxide
O_2	oxygen
USA	United States of America
HPLC	high performance liquid chromatograph
HPLC-MS	high performance liquid chromatograph coupled with mass spectometry
TFA	Trifluoroacetic acid
FLD	fluorescence detector
ANOVA	analysis of variance
AVG	aminoethoxyvinylglycine
RID	refractive index detector

ELSD	Evaporative Light-Scattering Detector
DAD	Photodiode array detector
NCBI	National Centre for Biotechnology Information
CTAB	Cetyl trimethyl ammonium bromide
RNA	ribonucleic acid
DNA	deoxyribonucleic acid
PCR	polymerase chain reaction
qPCR	quantitative polymerase chain reaction
qrtPCR	quantitative real time polymerase chain reaction
spp	species
viz	namely
ZR	zeatin riboside
ABA	abscisic acid
PA	phaseic acid
QToF	quadruple time of flight
CSPI	Centre for Science in the Public Interest, United States of America
FAO	Food and Agriculture Organization
FAOSTAT	Food and Agriculture Organization Statistics
HCL	hydrochloric acid
n.a	not available
lsd	least significance difference
P	probability
ABA-GE	abscisic acid –glucose ester

1 INTRODUCTION

1.1 Background

Sweet potato (*Ipomoea batatas* L.) is a very important crop in the tropics and it is one of the major staple food crops in West Africa. Ranked 4th in the world (Ray and Ravi, 2005) and 3rd in Sierra Leone, the sweet potato is mainly grown for its storage roots and is cultivated in more than 100 countries with an estimated global production of over 100 million metric tonnes (FAOSTAT, 2015). Global demand for this agricultural commodity is growing and meeting this requires continuous supply of the crop. However, this is limited by the perishability of the root after harvest as a result of shrivelling, sprouting and disease (Pedreschi et al., 2013) during storage. Fungi are one of the major microbial organisms associated with disease of stored sweet potatoes. Diseases such as surface rot caused by *Fusarium oxysporum*, root rot caused by *Fusarium solani* and soft rot caused by *Rhizopus stolonifer* (Kays and Kays, 1998; Cantwell and Suslow, 2013) have been reported. The degree of susceptibility of sweet potato to disease occurrence has been shown to be dependent on the cultivar and the storage environment (Holmes and Stange, 2002; Rees et al. 2003) and also piercing wounds on the surface of the root (Lewthwaite et al. 2013). Some of the fungi associated with disease incidence has the potential to induce the production of mycotoxins on the root which may be harmful to humans and animals (e.g. aflatoxins which are potent liver toxins and carcinogens) and therefore needs to be highlighted. Fungal species such as *Aspergillus* and *Fusarium* can infect the sweet potatoes pre-harvest and mycotoxin contamination can increase if storage conditions are poorly managed (Chulze, 2010).

In an attempt to address the postharvest problems on sweet potato, several storage technologies including Low temperature storage, Irradiation, the application of exogenous ethylene, etc. have been used. Fumigation of sweet potato with exogenous ethylene (10 µL L^{-1}) is gradually emerging as an effective means of supressing sprouting during storage (Cheema et al. 2013; Amoah et al. 2016). However, some adverse effects were observed as a result of the use of the gas viz: increased disease incidence, a rise in weight loss, increased respiration rates and increased metabolism of sugars.

The response of plants' tissue to ethylene is triggered by the activated complex resulting from the binding of ethylene to a receptor (Iqbal et al. 2013). This response then leads to a chain of reactions within the plant tissues which in turn results in a wide range of physiological changes. The regulation of ethylene in plant tissues triggers signals which elicit several physiological responses in activating target genes. Ethylene response factors (ERFs) constitute one of the largest transcription factors and are important regulators of low oxygen tolerance in plants as well as the biosynthesis of ethylene (Zhang et al., 2009; Cukrov et al., 2015). Ethylene insentitive 2 (EIN2) has also been shown to be a key player in the ethylene signalling pathway as its expression activates the response of the ethylene in plant tissues. The EIN2 protein accumulates as a result of ethylene treatment which is required for stabilization of the biosynthetic pathway process (Merchante et al., 2013).

Limiting the biosynthesis of ethylene has been carried out using many strategies including the application of aminoethoxyvinylglycine (AVG) (Cheema 2010), 1-methylcyclopropane (1-MCP) (Amoah and Terry, 2018) and CA storage. The AVG, 1-MCP and CA serve as ethylene binding receptors which actively repress the response of ethylene during its biosynthesis. The control of fungal growth and mycotoxin contamination in stored commodities using CA has also been reported as a possible strategy (Chulze, 2010). Lower levels of oxygen and elevated CO_2 could supress fungal development thereby consolidating the positive aspects of ethylene in sprout control by creating a hostile environment for fungi (pathogen-treatment interaction) to grow and consequently minimize the potential for mycotoxin contamination to arise. According to Chang and Kays (1981) weight loss and decay in stored sweet potatoes declined considerably under a CA treatment of 7 kPa O2 plus 2-3 kPa CO2. However, roots stored under less than 7 kPa O2 plus above 10 kPa CO2 developed an unpleasant flavour. Over a decade later, Kotecha and Kadam, (1998) reported that sweet potato held at 2-3 kPa CO_2 and 7 kPa O_2 incurred fewer losses in comparison to those stored in air. The authors further pointed out that an increased level of CO_2 above 10 kPa and an O_2 level below 7 kPa are associated with off flavour whereas O_2 levels between 2.5 and 5 kPa enhanced the accumulation of total sugars. Subsequently, Imahori et al. (2007), established that short term exposure of sweet potato to 1 kPa O_2 for seven days at 20˚C,

has the potential of prolonging the postharvest life of the roots in place of low temperature. These ranges of CA compositions suggested that used in the current study.

In as much as the adverse effects of ethylene could be inhibited by CA storage, its application has not been fully understood regarding sweet potato compared to potato (Khanbari and Thompson, 1996; Ma et al. 2010). This has led to piece meal information on the mechanics of CA storage systems (with or without ethylene supplementation) with respect to the quality of this important root crop during storage. Further research is therefore required to understand the actual molecular, biochemical and physiological mechanisms that take place in sweet potato stored under CA systems. Evidence on the exact relationship between gaseous composition of the storage atmosphere and quality degrading processes is yet to be established. Such information could help in the control and management of the storage conditions to inhibit quality decline in both the physiology and biochemistry of the stored roots. Despite the fact that storage treatments and temperature are important determinants of sweet potato quality, understanding the biology of the produce is equally vital in order to achieve a comprehensive strategy to address the problem. Therefore understanding the molecular processes regulating the biochemical dynamics during storage of the roots is of particular importance. This information may provide a greater understanding on the gene expression patterns in the different root tissues during storage as a result of the studied treatments.

1.2 Aim and objectives

To investigate the efficacy of controlled atmosphere and ethylene treatments on the physiological, biochemical, mycological and molecular dynamics of sweet potato during storage

Objective 1. To determine the effects of CO_2 and O_2 levels on the respiration rates, weight loss and spatial effects on dormancy and sprouting of sweet potato during storage.

Objective 2. To determine the spatial distribution of non-structural carbohydrates, polyphenols and plant growth hormones of sweet potato during storage under CA and ethylene conditions.

Objective 3. To determine the effects of storage of sweet potato in CA and ethylene on potential fungal and associated aflatoxin contamination across the different spatial gradients of the root

Objective 4. To investigate the molecular and metabolic mechanisms associated with the post-harvest quality of sweet potato during CA and ethylene storage.

1.3 Hypotheses

Objective 1:

H_o - The combined effect of controlled atmosphere and ethylene has little or no influence on the postharvest physiology of sweet potato during storage

H_i - The combined effect of controlled atmospheres and ethylene greatly influence the postharvest physiology of sweet potato during storage

Objective 2:

H_o: Spatial orientation has no influence on the biochemical dynamics during storage of sweet potato

H_i: Spatial orientation significantly influences the biochemical dynamics of sweet potato during storage

Objective 3:

H_o: Fungal growth and disease incidence cannot be controlled by storage of sweet potato under CA treatment

H_i: Fungal growth and disease incidence can be controlled by storage of sweet potato under CA treatment

Objective 4:

H_o: Controlled atmosphere and ethylene does not significantly affect the gene regulatory mechanism of metabolic activities in stored sweet potato

H_i: Controlled atmosphere and ethylene does influence the gene regulatory mechanism of metabolic activities in sweet potato during storage

1.4 Thesis structure

Table 1-1 Thesis plan and structure

Chapter	Objectives	Focus	Potential target
1	-	Background on sweet potato storage technologies	-
2	-	Review of controlled atmosphere and ethylene effects on stored sweet potato	*Food Science and Technology*
3	1 and 2	Physiological and biochemical mechanisms of stored sweet potato	*Postharvest Biology and Technology*
4	3	Fungal disease and aflatoxins production during sweet potato storage	*Food Microbiology/Fungal Diversity*
5	4	Molecular and metabolic flux in sweet potato during storage	*Journal of Experimental Botany*
6	-	Integrated discussion of thesis chapters	-
7	-	Conclusion and future work	-
8		Appendices	-

1.5 Conference papers

S. Sowe, S. Landahl, R. Tosetti, M. Anastasiadi and L. A. Terry, *Physiological and biochemical dynamics of sweet potato as affected by controlled atmosphere storage treatments*, VIII International Postharvest Symposium: Enhancing Supply Chain and Consumer Benefits - Ethical and Technological Issues, June 2016, Cartagena, Spain; paper accepted for publication in Acta Horticulturae

S. Sowe, A. Medina, R. Tosetti, S. Landahl and L. A. Terry, *Fungal development and potential mycotoxins on sweet potato stored under controlled atmosphere and ethylene conditions*, FRUTIC conference, February, 2017, Berlin, Germany

1.6 References

Amoah, R. S., Landahl, S., and Terry, L. A. (2016). The timing of exogenous ethylene supplementation differentially affects stored sweet potato roots. *Postharvest Biology and Technology*, 120, 92–102

Amoah, R.S. and Terry, L.A. (2018). 1-Methylcyclopropene (1-MCP) effects on natural disease resistance in stored sweet potato. *Journal of the Science of Food and Agriculture*, in Press

Cantwell, M. and Suslow, T. (2013). Sweet potato: Recommendations for maintaining postharvest quality. Department of Plant Science, University of California. [Available at http://postharvest.ucdavis.edu/pfvegetable/Sweetpotato ; accessed: 28th October, 2015]

Chang, L. A. and S. J. Kays. (1981). Effects of low oxygen storage on sweet potato roots. *Journal of American Society of Horticultural Science*, 106, 481–483.

Cheema, M.U. A., Rees, D., Westby, A. and Taylor, M. (2010). Hormonal Control of Sprouting of Sweet potatoes in Storage. *Acta Horticulturae*, 858, ISHS; 173-177

Cheema, M. U. A., Rees, D., Colgan, R. J., Taylor, M. and Westby, A. (2013). The effects of ethylene, 1-MCP and AVG on sprouting in sweet potato roots. *Postharvest Biology and Technology*, 85, 89–93.

Cukrov, D., Brizzolara, S. and Tonutti, P. (2015). Molecular and Metabolic Responses of Fruits to Low Oxygen Postharvest Stress. *Proceedings of the 11th International Controlled and Modified Atmosphere Research Conference*, (eds) Amodio, M.L. and Colelli, G., 3-7th June, 2013, Trani, Italy, *Acta Horticulturae,* 1071, Volume 1, pp. 37-43.

Delate, K. M., Brecht, J. K. and Coffelt, J. A. (1990). Controlled atmosphere treatments for control of sweet potato weevil (Coleoptera: *Curculionidae*) in stored tropical sweet potatoes. *Journal of Economic Entomology*, 82, 461–465.

FAOSTAT (2015), Food and Agriculture Organisation database on Agriculture; available at: http://faostat3.fao.org (accessed: 22nd January, 2017)

Imahori, Y., Kishioka, I., Uemura, K., Makita, E., Fujiwara, H., Nishiyama, Y. and Chachin, K. (2007). Effects of Short-term Exposure to Low Oxygen Atmospheres on Physiological Responses of Sweet potato Roots. *Journal of the Japanese Society for Horticultural Science*, 76(3), 258–265.

Iqbal, N., Trivellini, A., Masood, A., Ferrante, A. and Khan, N. A. (2013). Plant Physiology and Biochemistry Current understanding on ethylene signalling in plants : The influence of nutrient availability. *Plant Physiology and Biochemistry*, 73, 128–138.

Khanbari, O. S. and Thompson, A.K. (1996). Effect of controlled atmosphere, temperature and cultivar on sprouting and processing quality of stored potatoes. *Potato Research*, 39, 523-531.

Ma, Y., Wang, Q., Gyunghoon Hong, G. and Cantwell, M. (2010). Reassessment of treatments to retard browning of fresh-cut Russet potato with emphasis on controlled atmospheres and low concentrations of bisulphite. *International Journal of Food Science and Technology*, 45, 1486–1494.

Merchante, C., Alonso, J. M. and Stepanova, A. N. (2013). Ethylene signaling: Simple ligand, complex regulation. *Current Opinion in Plant Biology*, 16(5), 554–560.

Pedreschi, R., Lurie, S., Hertog, M., Nicolaï, B., Mes, J. and Woltering, E. (2013). Post-harvest proteomics and food security. *Proteomics*, 13, 1772–1783.

Ray, R.C. and Ravi, V. (2005). Post-harvest spoilage of sweet potato and its control measures. *Critical Review of Food Science and Nutrition* 35, 623-644.

Zhang, Z.J., Zhang, H.W., Quan, R.D., Wang, X.C. and Huang, R.F. (2009), Transcriptional Regulation of the Ethylene Response Factor LeERF2 in the Expression of Ethylene Biosynthesis Genes Controls Ethylene Production in Tomato and Tobacco. *Plant Physiology*, 150, 365-377

2 FUTURE SWEET POTATO STORAGE IN WEST AFRICA: A REVIEW

S. Sowe and L.A. Terry*

Plant Science Laboratory, Cranfield University, Bedfordshire, MK43 0AL, United Kingdom

*Corresponding author - E-mail: l.a.terry@cranfield.ac.uk ; Tel: +44 (0)1234 750111

2.1 Introduction

Sweet potato (*Ipomoea batatas*, Lam) is a very important nutritious root crop, ranked 4th in the world and the 2nd most important root crop after Cassava (Ray and Ravi, 2005; Thottappily, 2009). The crop is cultivated in more than 100 countries in the tropical and sub-tropical regions of the world, with an estimated global production of over 100 million metric tonnes (FAOSTAT, 2015). The sweet potato is ranked the healthiest root vegetable by nutritionists at the Centre for Science in the Public Interest, Washington DC (CSPI, 2013). In Sierra Leone, sweet potato is the third most important food commodity after the staple food crop rice and cassava (Table 2-1). The increasing awareness of sweet potato as a cheap and valuable source of pro-vitamin A which plays an important role in preventing blindness in children, its superior advantage in terms of calorific value - 351.30 kcal compared to other root crops and cereals (ARS, 2008; Maxwell, 2014) has contributed to a gradual increase in the consumption of the crop in rural and urban households in West Africa. Consequently, more farmers are getting engaged in the production of the crop because it is a profitable venture - a net benefit of about Le 3,450,380/ha (GBP 479.22/ha) has been reported from marketing of sweet potato in Sierra Leone (Crop Production Guidelines for Sierra Leone, 2005).

Sweet potato contains biologically active phytochemicals, minerals, vitamin C, beta-carotene, dietary fibre, polyphenols and also anti-oxidant and anti-diabetic properties which offer the crop benefits for use as a functional food ingredient (Brinley et al. 2008; Ezekiel et al., 2013). However, the contents of these metabolites mentioned above may be completely or partially lost during storage and/or processing (e.g. baking, frying, cooking); therefore appropriate modern storage and processing methods must be considered in order to reduce loss of these compounds.

Table 2-1 Characteristics of sweet potato varieties in Sierra Leone

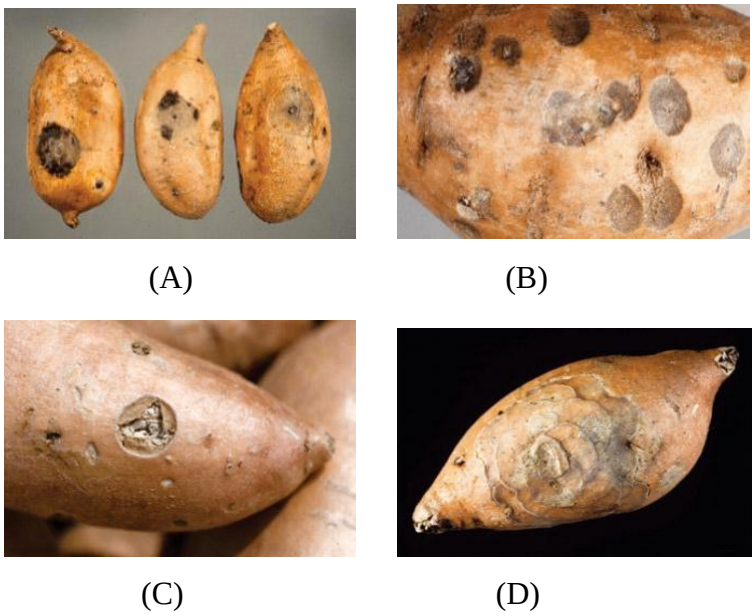
Characteristics		Cultivars			
		SLIPOT-1 (Clone82/123R)	SLIPOT-2 (Clone82/123W)	SLIPOT-3 (Clone82/144)	SLIPOT-4 (Clone 84/16)
Plant type	Length of main vine	Spreading (1.5-2.5m)	Spreading (1.5-2.5m)	Semi-erect (0.75-1.5m)	Semi-erect (0.75-1.5m)
Leaf	Leaf outline	Triangular	Triangular	Triangular	Lobed
Vine	Pigmentation	Green	Green	Mostly purple	Green
Storage root	Shape	Round elliptic	Round elliptic	Round elliptic	Round
	Skin colour	Purple-red	White	Purple-red	Off-white
	Flesh colour	White	White	Dark cream	White
	Dry matter %	High (28)	High (28)	High (29)	High (29)
	Texture-boiled root	Soft and creamy	Soft and creamy	Soft and creamy	Soft and creamy
	Taste –boiled root	Sweet	Sweet	Very sweet	Sweet
Reaction to diseases	Scab	Resistant	Resistant	Resistant	Resistant
	Virus complex	Resistant	Resistant	Moderately susceptible	Resistant
Reaction to Pests	Weevil	Susceptible	Susceptible	Escape	Susceptible
Maturity		3.5-4.0 months	3.5 months	4.0 months	3.5 months
Tuber Yield Potential		12t/ha	15t/ha	10t/ha	10t/ha

Source: Crop production guidelines for Sierra Leone (2005)

2.2 Sweet potato storage

The roots of sweet potato are generally susceptible to perishability post-harvest under tropical conditions resulting in a much shorter storage life (Fowler and Stabrawa, 1992). This problem can be mitigated if the fresh roots are properly handled through curing, drying and storage. Appropriate storage is a very important unit operation along the sweet potato value chain in order to achieve reduced senescence, reduced metabolic activity and reduced water loss, reduced sprouting and disease incidences (Figure 2-1) coupled with ensuring adequate supply for food and feed throughout the year. The quality of sweet potato during storage in turn also plays a significant role in enhancing diverse utilization of the roots into various value-added products such as starch, flour, soft drinks, confectioneries, pharmaceuticals etc. According to Picha (1987), sweet potato roots stored at 13-15^{o}C and under high relative humidity, can be kept for up to one year. A much recent report by Hayma (2003) reported that roots of sweet potato can remain marketable for as long as 13 months with good quality when properly cured and held undisturbed at storage conditions of 13˚C and 85 - 90% relative humidity. In developing countries, including Sierra Leone, traditional technologies of sweet potato storage have been in existence for decades and most of those technologies are still been used. Also sweet potato storage at subsistence level is a rare practice, except for brief periods of time (Karuri and Hagenimana, 1995). This might be due to the fact that the produce can be harvested all year round and is therefore available in its fresh condition on a continuous basis for marketing throughout the year. Despite this, huge postharvest losses using traditional storage methods have reported in sub-Saharan Africa viz: storage in sacks, jute bags, clamps, in mounds and/or perforated baskets with losses ranging from 20-40 % (Sesay et al., 1989). Tortoe et al. (2010) reported that tropical storage of sweet potatoes in clamps results in a prolonged storage life as compared to pit storage. This contradicts reports (DFID - Crop Post-Harvest Program) that the best method for long term storage of sweet potatoes in the tropics is pit storage without grass lining. In addition, Hall and Devereau (2000) reported that with the methods of traditional pits and/or clamps, sweet potatoes can be stored for up to 3 - 4 months if careful selection of roots is taken into consideration. However, studies on the effects of these traditional storage technologies have been on the general effects and remain confusing and contradictory. Also there is as yet no report on the impact of traditional

storage on the mechanisms of biochemical and physiological changes in sweet potato. Modern sweet potato storage technologies: Low temperature and controlled atmosphere (CA) storage (Delate et al., 1990), the use of exogenous ethylene (Cheema et al., 2013; Amoah et al., 2016) and the use of chemicals such as Chloropropham (CIPC), have been employed to address the problem of deterioration during storage. This review will focus mainly on the effects controlled atmosphere (CA) storage with and without the supplementation of exogenous ethylene in controlling postharvest physiological deterioration as well as the associated biochemical mechanisms during storage of sweet potato.



(A) (B)

(C) (D)

Figure 2-1 Common postharvest diseases that affect stored sweet potatoes – (A) and (B) Black rot caused by *Ceratocystis fimbriata*, (C) Fusarium surface rot, characterized by sunken, scalloped-edge rings on the root surface and (D) Circular spot, caused by the fungus *Sclerotium rolfsii*. Lesions are unusually circular and the centre of the spot typically cracks (Photo by G. Holmes), Source: Postharvest handling of sweet potatoes (Published by North Carolina Cooperative Extension Service 2003)

2.3 The biosynthesis of ethylene in plant tissues

Ethylene is a gaseous plant hormone at room temperature, which regulates a multitude of plants' growth and development processes under optimal and stressful environment (Iqbal et al., 2013).The level of ethylene concentrations in plant tissues is dependent on the influence of temperature, hypoxia, mechanical injury, decay and disease (Chang and Bleecker, 2004). The biosynthesis of ethylene involves two major enzymes: 1-

aminocyclopropane-1-carboxylase synthase (ACS) and 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase (ACO) and the process occur through a simple biochemical pathway. The ACS is a multigene family enzyme and therefore response to external stresses and gene expression during growth and development of plant tissues may vary. The process of ethylene biosynthesis involves the conversion of methionine to S-adenosylmethionine (AdoMet) which is a substrate, by S-adenosylmethionine synthetase (Jouyban, 2012). The susbstrate is then converted into ACC (1-aminocyclopropane-1-carboxylic acid) and 5′-deoxy-5′methylthioadenosine (MTA) by the enzyme ACS. This process is based on the Yang cycle and is regarded as the rate limiting step. ACC is then oxidised by the enzyme ACC oxidase (ACO), resulting in the production of ethylene, CO_2 and cyanide (Chang and Bleecker, 2004) as illustrated in Figure 2-2 below.

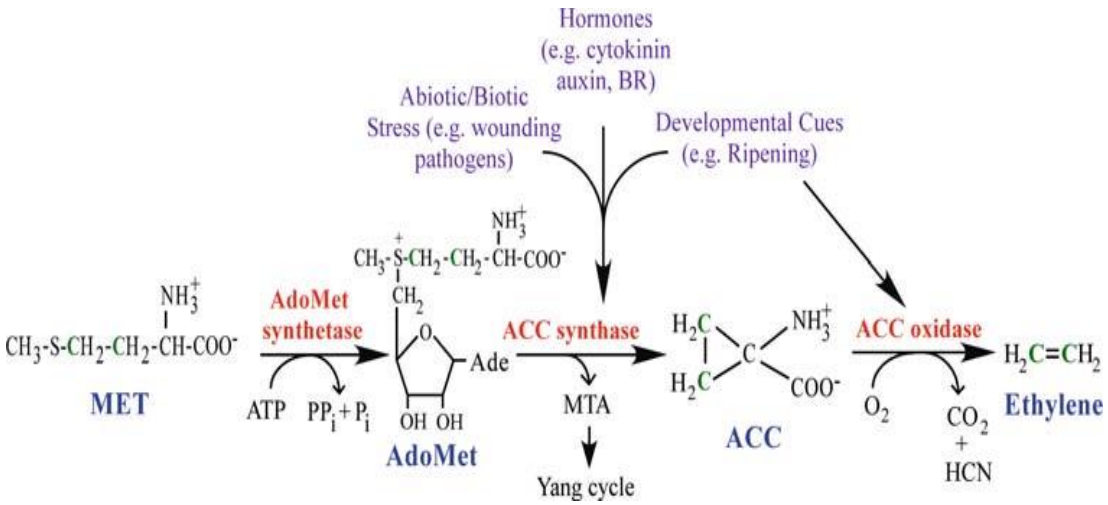


Figure 2-2 Ethylene biosynthetic pathway. The enzymes catalysing each step are shown above with arrows; AdoMet: S-adenosyl-methionine; Met: methionine; ACC: 1-aminocyclopropane-1-carboxylic acid; MTA: methylthioadenine. Inputs that regulate the enzymes are shown above the pathway, either via a transcriptional or post-transcriptional mechanism. See text for further details (Source: Argueso et al. 2007).

Limiting the biosynthesis of ethylene has been carried out using many strategies including the application of aminoethoxyvinylglycine (AVG) (Cheema 2010) and CA storage. The regulation of ethylene in plant tissues triggers signals which elicit several physiological responses in activating target genes. According to Iqbal et al. (2013), plants' tissue response to ethylene is triggered by the activated complex resulting from the binding of ethylene to a receptor. This response then leads to a chain of reactions

within the plant tissues which in turn results in a wide range of physiological changes. Ethylene response factors (ERFs) which are located "downstream of the ethylene signalling pathways" constitute one of the largest transcription factors and are important regulators of low oxygen tolerance in plants (Cukrov et al., 2015). They also play a key role in regulating the biosynthesis of ethylene (Zhang et al., 2009).

Although the level of ethylene production can be affected by other plant growth regulators such as auxins, abscisic acid (ABA), gibberellins and cytokinins, it is worth noting that the gas can affect its own biosynthesis by auto-stimulation or auto-inhibition (Argueso et al. 2007). The cross-talk between the signalling pathways of ethylene and the other plant growth regulators is the main factor responsible for the dependent relationships in their biosynthesis (Jouyban, 2012). In an effort to unveil the molecular aspects of ethylene's functions in plant tissues, Chang et al. (2013), used the ChIP-Seq technique to identify the regions of the DNA that the plant protein EIN3 binds to in the model plant *Arabidopsis thaliana*. Simultaneously, the authors used genome-wide mRNA sequencing to identify the genes that showed altered transcription. The study indicated that "ethylene-induced transcription occurs in temporal waves regulated by EIN3, suggesting distinct layers of transcriptional control. The protein EIN3 binding was found to modulate a multitude of downstream transcriptional cascades as well as integrating numerous connections between most of the hormone mediated growth response pathways.

2.4 The effects of exogenous ethylene on sweet potato during storage

The root of sweet potato, like the potato tuber produces very small amounts of ethylene, *c.a.* 0.1 µL/kg·hr (Chope and Terry, 2008; Cantwell and Suslow, 2013). Both endogenous and exogenous ethylene has been shown to affect quality changes such as disease, sprouting, weight loss and nutrient losses, in the root (Saltveit, 1999). The extent of this effect in the sweet potato is yet to be fully established even though the small amounts of ethylene produced by the roots may make a substantial change unlikely. Cheema et al. (2013) and Amoah et al. (2016) found that sprout suppression by ethylene (10 µL L^{-1}) is associated with increased respiration rates and increased sugar metabolism. The negative effects of ethylene such as microbiological decay and weight loss in potato (Foukaraki et al., 2011) and sweet potato (Cheema et al., 2013;

Amoah and Terry, 2018) can be evaded by the use of antagonistic chemicals such as the 1-Methylcyclopropane (1-MCP). In the case of potato, Foukaraki et al. (2011) reported sprout inhibition and reduced sugar accumulation in potato *cv*. Marfona as a result of ethylene (10 µL L^{-1}) storage. Contrasting reports about ethylene have been made; some authors (Coleman, 1998) described it as the most effective dormancy release agent whilst others (Foukaraki et al., 2014) described it as an effective sprout suppressant when applied continuously at 10 µL L^{-1}. In a study to understand the mechanisms of sprouting in potato cultivars using UV-C radiation, Cools et al. (2014) reported effective suppression of sprout length and incidence with a treatment dose of 5–20 kJ m^{-2} in a range of cultivars with no adverse effects on the quality. In 2016, Amoah et al. reported that ethylene supplementation after dormancy break was as effective as uninterrupted ethylene treatment in mitigating sprout growth on the stored sweet potatoes over time.

Ethylene, like abscisic acid (ABA) has been shown to play an important role in dormancy initiation in potato, although the interaction between these two plant hormones is unclear (Suttle, 2007). Prange et al. (1998) had suggested that exogenous ethylene shortens or delays the dormancy period in potato depending on the duration and concentration of application. More than a decade later, Hartmann et al. (2011) reported increased level of ethylene production with the onset of sprouting and that ethylene-associated signals negatively influence the growth of sprouts in potato. The role of ABA in controlling dormancy and sprouting in potato and bulbs is well documented (Sonnewald and Sonnewald, 2014; Ordaz-Ortiz et al., 2015; Foukaraki et al., 2016). According to Destefano-Beltran et al., (2006), the levels of ABA are highest in deeply dormant potato tubers but decrease during the period of storage. The authors pointed out a correlation between the declines of ABA content during the dormancy period with increased expression of ABA 8′-hydroxylase. Ordaz-Ortiz et al. (2015) measured the amount of plant hormones in the skin and flesh of two processing potato cvs: Sylvana and Ruset Burbank stored at 6ºC for 30 weeks. An inverse relationship between ABA content and storage duration was observed, which supports the link of ABA with potato tuber dormancy progression. Abscisic acid – glucose ester (ABA-GE) seems to accumulate in the skin of potatoes throughout storage time and Ordaz-Ortiz et al. (2015) added that the content of ABA-GE "do not always vary in parallel to the

change in ABA levels, suggesting that conjugation may be regulated differently in particular tissues and under specific conditions". There could possibly be a similar pattern with regards to sweet potato on the role of ABA in controlling dormancy and sprouting. ABA and exogenous ethylene have been shown to influence the mechanism of sprout suppression in sweet potato (Amoah et al., 2016).

Figure 2-3 Sprouting in sweet potato due to poor curing or storage conditions (photo by G. Holmes); Source: Postharvest handling of sweet potatoes (Published by North Carolina Cooperative Extension Services 2003)

Dormancy is a phenomenon generally regarded as the period of relative inactive physiological activity in plant tissues. The end of dormancy marks the onset of gradual growth and development of buds and subsequently sprouting (Rees et al., 2003; van Oirschot et al., 2003; Pringles et al., 2009). Earlier reports by Ravi and Aked (1996) suggested a variation in the postharvest storage life of crops and cultivars due to their corresponding period of dormancy. The process of dormancy can be categorised into three phases *viz:* Endodormancy, Paradormancy and Ecodormancy. Endodormancy refers to the innate rest period and is dependent on the genotype and the inner physiological factors whilst paradormancy is affected by physiological factors. Ecodormancy on the other hand is a rest period determined by external factors such as temperature and relative humidity.

Contrasting views have emerged over the past decades regarding the physiological and biochemical mechanisms underlying the dormancy phenomenon in plant materials. Champagnat (1989) suggests changes in the sink activity, water status in the buds and

hormonal balance as the major factors controlling the process whilst Petel et al. (1992) suggested an exchange of sink power between the initial bud and the surrounding tissues. During dormancy, the root acts as a sink organ by accumulation of starch during the bulking period whilst the bud remains dormant. On the other hand, at dormancy break the root acts as a nutrient supply organ to the growing buds. The promotion of sprouts growth in potato by gibberellins occurs by stimulating DNA and RNA synthesis of (Rossouw, 2008). A report by Cheema et al. (2010) also suggested a similar pattern in sweet potatoes. The authors further explained that the levels of gibberellic acids (GAs) (GA1, GA19 and GA20) increased as sprout growth became more vigorous. Increased levels of GAs must therefore be controlled to maintain the quality of sweet potato during storage. Unlike ethylene and ABA, gibberellins (GA) are associated with dormancy break and sprout promotion in roots and tubers. Rentzsch et al. (2012) confirm the role of gibberellins as a dormancy release agent in potato by treating with bioactive GA, which resulted in the termination of dormancy and the stimulation of bud outgrowth. GA1 which is the main bioactive endogenous GA in potato, GA19 and GA20 have been shown to remain unchanged during the dormancy period. The content of this compound however, increases with the onset of sprouting (Sonnewald and Sonnewald, 2014).

Cytokinins have also been shown to interact with other plant growth regulators including ethylene and ABA in regulating dormancy and germination (Kucera et al., 2005) and also in the activation of target genes in plant tissues. Zeatin riboside (ZR) is the most active naturally occurring cytokinin and its content and sensitivity have been linked to the control of dormancy and sprouting in potato (Suttle, 2008). Amoah et al., (2016) discussed the regulation of sprout growth as a result of the cross talk between exogenous ethylene and ZR biosynthesis in sweet potato. Also according to Amoah et al. (2016) a similar cytokinin-sequestering protein (cytokinin riboside phosphorylase) in potato which down-regulated tissue sensitivity to cytokinins is possibly induced in sweet potato in the presence of exogenous ethylene which forms a complex with ZR and inhibits its action until ethylene is removed. However the mode of interaction of these hormones in bringing the explained phenomenon is yet to be understood. Despite many studies in investigating the mechanics of dormancy in plant materials, there is not a universal consensus regarding its full definition. The association of dormancy with

reduced metabolic activity, external environmental conditions and the endogenous variables is not fully understood. Further investigation is therefore required to understand the relationship between the external and internal physiological factors that take place and how they trigger these two processes.

2.5 Controlled Atmosphere Storage

Limiting the biosynthesis of ethylene has been carried out using many strategies including the use of CA storage. In as much as a wide range of storage technologies have been used to extend the postharvest life of sweet potato, CA storage has not been exploited as much as it - should. This storage technology in many ways is normally applied as a supplement to, and not as a substitute for, temperature and relative humidity management. Controlled atmosphere storage of agricultural commodities was first introduced by Franklin Kidd and Cyril West in 1910 (FGN, 2011). These two scientists who were working at the Low Temperature Research Station at Cambridge, United Kingdom, studied the respiration and ripening of fruits as affected by temperature, CO_2 and O_2. This subsequently led to the introduction of the first ever commercial gas storage technique for apples in 1929. A year later, the commercial potential of a CA storage system was realized which then resulted in the rapid expansion of this technology for apples and pears. Over two decades later in the 1950s, the success of this storage technology spread to other parts of the world, including the United States where the first commercial CA storage facility was created for apples and the technology for these commodities has expanded ever since. According to Chong et al. (2013), CA storage systems can be classified as closed or open. The closed systems involve a sealed storage vessel where the gas concentration within the container is solely determined by the initial gas composition and produce respiration. On the other hand, the open systems involve modifying the storage atmosphere by purging the vessel with nitrogen gas.

Some of the benefits of CA storage include: maintenance of natural disease resistance, decreased pathogen metabolic rate and decreased disease incidence (Prange et al., 2006). Elevated levels of CO_2 and reduced O_2 levels have been reported to alter primary and secondary metabolism of stored fresh produce and the process in turn affects the amount of energy produced to support the metabolic rate (Kanellis et al., 2009). Despite

these commercial practicalities, the mode of action in ultra-low oxygen and high CO_2 in fresh produce is not well understood. The dynamic nature of this metabolism of horticultural produce makes SCA an unlikely option to attain optimal storage conditions; hence the need for dynamic CA system. In addition, research on the use of CA systems has been on the general effects on lowering the respiration rates of stored produce with less emphasis on the quality loss with respect to the conditions applied (Nicolaï et al., 2009). Also, CA reduces the pathogen respiration rate, and can maintain natural disease resistance. However, the development of off-odours, off-flavours and physiological disorders has also been reported as a result of poor management of CA storage system (Falagan and Terry, 2017).

2.5.1 Applications of controlled atmosphere storage on sweet potato

The application of CA storage on sweet potato is not as widely practised as in apples, pears, onions and even potato tubers. This has led to piece meal investigations on the full benefits CA storage has to offer to this important root crop. Due to the similarities in terms of the dormancy and sprouting phenomenon, the roots of sweet potato exhibit similar properties to potato and therefore may also have some similarities in terms of response to various gas compositions. According to Kotecha and Kadam, (1998) sweet potato held at 2-3 kPa CO_2 and 7 kPa O_2 incurred fewer losses in comparison to those stored in air. The authors further pointed out that an increased level of CO_2 above 10 kPa and an O_2 level below 7 kPa are associated with off flavour whereas O_2 levels between 2.5 and 5 kPa enhanced the accumulation of total sugars.

Storage pests such as insects (e.g. weevils) are generally killed more rapidly by increased CO_2 than they are by the lack of oxygen. However, levels of 40-60 kPa CO_2 and 2-4 kPa O_2 at 25˚C during storage have been reported to cause increased decay and off-flavour in the roots of sweet potato as well as 100% mortality in adult weevils (Delate et al., 1990). Subsequent report by Mitcham et al. (2003) confirms the efficacy of CA storage in the control of insects in fresh horticultural produce. Controlled atmospheres of 60 kPa CO_2, 20 kPa O_2, 20 kPa N_2 and 20 kPa CO_2, 40 kPa O_2, 40 kPa N_2 in combination with ethylene have been reported to decrease the ABA content in potato within 24h (Coleman, 1998) regardless of previous storage temperature.

Khanbari and Thompson (1996) have reported a complete sprout inhibition, low weight loss and maintenance of healthy skin in potato cultivars stored at CA regimes of 9.4 kPa and 6.4 kPa CO_2 and 3.6 kPa O_2 at 5°C for 25 weeks. The authors reported a maintained healthy skin and no indication of sprouting for a further period of 20 weeks when the tubers were stored in air at the same temperature. However despite the benefits of extended postharvest life and physical quality, crisps derived from these stored potato tubers were characterized with dark fry colour which falls short of the required industry standard. The use of CA with respect to the control of dormancy and sprouting in root and tuber crops is yet to be fully investigated. Cantwell and Suslow (2013) also reported a non-existent commercial use of CA for sweet potato and also a lack of documented information on the response of increased CO_2 levels on the quality of the roots.

Information on the mechanics of CA storage systems with respect to the quality of sweet potato is limited. Additional research is therefore required to understand the actual molecular, biochemical and physiological mechanisms that take place in sweet potato stored under CA systems. Evidence on the exact relationship between gaseous composition of the storage atmosphere and quality degrading processes is yet to be established; such information could help in the control and management of the storage conditions to inhibit quality decline in both the physiology and biochemistry of the stored product.

2.6 Conclusions

Designing a one size fits all CA condition is the greatest challenge faced by the application of this storage technology. The CA requirements for fresh produce vary from one commodity to the other, from one cultivar of the same commodity to the other. Better understanding on the main variables that controls the quality of stored produce viz: storage duration, temperature, humidity, and O_2, CO_2, and ethylene levels is needed to develop models that could measure product response to the changes that takes place during storage and how it affects the quality.

The application of exogenous ethylene during storage of sweet potato needs to be further investigated to actually understand the biological mechanisms surrounding its effectiveness regarding shelf life and storage life extension. Despite the fact that the sweet potato produces small amounts of endogenous ethylene (*c.a.* 0.1 µL/kg·hr), the

interaction of exogenous ethylene and the antagonistic agents such as CA needs to be evaluated to determine the possible combined effect towards counteracting the adverse effects of exogenous ethylene.

2.7 Acknowledgements

My sincere thanks and appreciation go to the Commonwealth scholarship commission for their support in providing me the opportunity to have this great experience. I would also like to thank members of the plant science group for their technical assistance.

2.8 References

Agricultural Research Service (2008), Sweet potato out-yields corn in ethanol production study. Agricultural Research Service (ARS) Report, USDA; http://www.ars.usda.gov/is/pr/2008/080820.html (Acessed: 27th November, 2015)

Amoah, R. S., Landahl, S., and Terry, L. A. (2016). The timing of exogenous ethylene supplementation differentially affects stored sweet potato roots. *Postharvest Biology and Technology*, 120, 92–102

Amoah, R.S. and Terry, L.A. (2018). 1-Methylcyclopropene (1-MCP) effects on natural disease resistance in stored sweet potato. *Journal of the Science of Food and Agriculture*, in Press

Argueso, C. T., Hansen, M. and Kieber, J. J. (2007). Regulation of ethylene biosynthesis. *Journal of Plant Growth Regulation*, 26, 92–105.

Cantwell, M. and Suslow, T. (2013), Sweet potato: Recommendations for maintaining postharvest quality. Department of Plant Science, University of California. [Available at http://postharvest.ucdavis.edu/pfvegetable/Sweetpotato ; accessed: 28th October, 2015]

Centre for Science in the Public Interest (CSPI), Washington DC (2013). Available online at http://.www.foodreference.com. (Accessed: February, 2015).

Champagnat, P. (1989). Rest and activity in buds of trees. *Annales des Sciences Forestiries*, 46, 9-26.

Chang, C. and Bleecker, A. B. (2004), Ethylene Biology. More than a Gas. *Plant Physiology*, 136, 2895-2899

Chang, K. N., Zhong, S., Weirauch, M. T., Hon, G., Pelizzola, M., Li, H., Huang,S.C., Schmitz, R.J., Urich, M.A., Kuo, D., Nery, J.R., Qiao, H., Yang, A., Jamali, A., Chen, H., Ideker, T., Ren, B., Ziv, B.J., Hughes, T.R. and Ecker, J. R. (2013), Temporal Transcriptional Response to Ethylene Gas Drives Growth Hormone Cross-Regulation in Arabidopsis. *eLIFE*, 2:e00675, 1–20.

Cheema, M. U. A., Rees, D., Colgan, R. J., Taylor, M. and Westby, A. (2013). The effects of ethylene, 1-MCP and AVG on sprouting in sweet potato roots. *Postharvest Biology and Technology*, 85, 89–93

Chong, K. L., Peng, N., Yin, H., Lipscomb, G. G., and Chung, T. S. (2013). Food sustainability by designing and modelling a membrane controlled atmosphere storage system. *Journal of Food Engineering*, 114(3), 361–374.

Chope, G.A. and Terry, L.A. (2008). The role of abscisic acid and ethylene in onion bulb dormancy and sprout suppression. *Stewart Postharvest Review*, 4 (2), p.7

Coleman, W. K. (1998), Carbon Dioxide, Oxygen and Ethylene effects on potato tuber dormancy release and sprout growth. *Annals of Botany*, 82, 21–27

Cools, K., Alamar, del Carmen, M., and Terry, L. A. (2014). Controlling sprouting in potato tubers using ultraviolet-C irradiance. *Postharvest Biology and Technology*, 98, 106–114.

Crop Production Guidelines for Sierra Leone (2005), p. 30-36

Cukrov, D., Brizzolara, S. and Tonutti, P. (2015). Molecular and Metabolic Responses of Fruits to Low Oxygen Postharvest Stress. *Proceedings of the 11th International Controlled and Modified Atmosphere Research Conference*, (eds) Amodio, M.L. and Colelli, G., 3-7th June, 2013, Trani, Italy, *Acta Horticulturae*, 1071, Volume 1, pp. 37-43.

Delate, K. M., Brecht, J. K. and Coffelt, J. A. (1990). Controlled atmosphere treatments for control of sweet potato weevil (Coleoptera: *Curculionidae*) in stored tropical sweet potatoes. *Journal of Economic Entomology*, 82, 461–465.

Destefano-Beltran L., Knauber D., Huckle L. and Suttle J.C (2006). Effects of postharvest storage and dormancy status on ABA content, metabolism, and

expression of genes involved in ABA biosynthesis and metabolism in potato tuber tissues. *Plant Molecular Biology*, 61, 687–697

Ezekiel, R., Narpinder, S., Shagun, S. and Amritpal, K. (2013). Beneficial phytochemicals in potato –A review. *Food Research International;* **50**, 487-496.

FAOSTAT (2015), Food and Agriculture Organisation database on Agriculture; available at: http://faostat3.fao.org (accessed: 22nd January, 2017)

Foukaraki, S.G.; Chope, G.A. and Terry, L.A. (2011). 1-MCP application before continuous ethylene storage suppresses sugar accumulation in the UK-grown potato cultivar 'Marfona'. IV International Conference, Postharvest Unlimited, *Acta Horticulturae*, 945.

Foukaraki, S. G., Cools, K., Chope, G. A., and Terry, L. A. (2014). Effect of the transition between ethylene and air storage on post- harvest quality in six UK-grown potato cultivars. 89, 599–606.

Foukaraki, G., Cools, K. and Terry, L. A. (2016). Differential effect of ethylene supplementation and inhibition on abscisic acid metabolism of potato (*Solanum tuberosum*, L.) tubers during storage, *Postharvest Biology and Technology*, 112, 87–94.

Fowler, M. H. and Stabrawa, A. M. (1992), A rapid appraisal of sweet potato marketing in Uganda. In: Scott, G., Ferguson, P.I. and Herrera, J. E(eds). http//www.cipotato.org (accessed 12th December, 2014)

Fruit Growers News (FGN), edited by Derreck Sigler – 2011. Available at: http://www.fruitgrowersnews.com/index.php./magazine/article - accessed: 9th April, 2015

Hall, A. J. and Devereau, A. D.(2000). Low-cost storage of fresh sweet potatoes in Uganda: lessons from participatory and on-station approaches to technology choice and adaptive testing. *Outlook on Agriculture*, 29**,** 275-282.

Hartmann, A., Senning, M., Hedden, P., Sonnewald, U. and Sonnewald S. (2011). Reactivation of meristem activity and sprout growth in potato tubers require both cytokinin and gibberellin. *Plant Physiology*, 155,776–796

Hayma, J. (2003), The storage of tropical agricultural products. Agrodak 31, fourth edition. Agromisa Foundation, Wageningen.ISBN:90-77073-60-4; NUGI:835; p.7-42

Iqbal, N., Trivellini, A., Masood, A., Ferrante, A. and Khan, N. A. (2013). Plant Physiology and Biochemistry Current understanding on ethylene signalling in plants : The influence of nutrient availability. *Plant Physiology and Biochemistry*, 73, 128–138.

Jouyban, Z. (2012), Ethylene Biosynthesis. *Technical Journal of Engineering and Applied Sciences*, 1(4), 107–110

Kanellis, A.K., Tonutti, P. and Perata, P. (2009) 'Biochemical and Molecular Aspects of Modified and Controlled Atmospheres', in Yahia, E.M (ed), *Modified and Controlled Atmospheres for the Storage, Transportation and Packaging of Horticultural Commodities*. CRC Press: Taylor & Francis Group, p. 553-563.

Karuri,E.G. and Hagenimana. V. (1995), Use of ambient conditions and sawdust in storage of sweet potato (*Ipomoea batatas* (L) roots in Kenya and Zimbabwe. *Journal of Agricultural Research,* 33, 83-91.

Khanbari,O.S. and Thompson, A.K. (1996). Effect of controlled atmosphere, temperature and cultivar on sprouting and processing quality of stored potatoes. *Potato Research*, 39, 523-531.

Kotecha, P.M. and Kadam, S.S. (1998), "Sweet Potato" in Salaunkle, D.K. and Kadam, S.S. (eds), *Handbook of Vegetable Science and Technology: Production, Composition, Storage and Processing*. CRC Press: Marcel Dekker, Inc; pp. 71-93 (Available at http://books.google.co.uk – accessed: 10th April, 2015)

Kucera, B., Cohn, M.A. and Leubner-Meetzger, G. (2005). Plant hormone interactions during seed dormancy release and germination. *Seed Science Research*, 15, 281-307.

Maxwell, M. (2014), North Carolina Sweet Potato Acreage Climbs. *News and insight for North America's fresh produce buyers*; In America Fruit. Available at: http://www.fruitne t.com/ame ricafruit/article /1474/parts-of-san-die go-quarantine d-as-psyllid-countm-ounts (Accessed: November, 2014)

Mitcham, E. J., Lee, T., Martins, A., Zhou, S. and Kader, A. A. (2003). Summary of Controlled Atmospheres for Arthropod Control on Fresh Horticultural Perishables. *Acta Horticulturae*; 600, 741-745.

Nicolaï, B.M., Hertog, Maarten L.A.T.M., Tri Ho, Q., Verlinden, B.E. and Verboven, P. (2009), 'Gas Exchange Modelling', in Yahia, E.M (ed), *Modified and Controlled Atmospheres for the Storage, Transportation and Packaging of Horticultural Commodities*. CRC Press: Taylor & Francis Group; p. 93-107

Ordaz-ortiz, J., Foukaraki, S., and Terry, L. A. (2015). Assessing temporal flux of plant hormones in stored processing potatoes using high definition accurate mass spectrometry. *Horticultural Research*, 2, 1–8.

Petel, G., Lafleuried, J., Dauphin, G. and Gendraud, M. (1992). Cytoplasmic pH and Pleomalemma ATPase activity of parenchyma cells during the release of dormancy of Jerusalem artichoke tubers. *Plant Physiology and Biochemistry*, 30, 379-382.

Picha, D.H. (1987), Carbohydrate changes in sweet potatoes during curing and storage. *Journal American Society of Horticultural Science*,112, 89-92.

Prange, R.K., Delong, T.A., Daniels-Lake, B.J. and Harrison, P.A. (2006). Controlled Atmosphere related disorders of fruits and vegetables. *Stewart Postharvest Review*, 5(7), 1-11

Prange, R.K., Kalt, W., Daniels-Lake, B.J., Liew, C.L., Page, R.T., Walsh, J.R., Dean, P. and Coffin, R. (1998). Using ethylene as a sprout control agent in stored 'Russett Burbank' potatoes. *Journal of the American Society of Horticultural Science*; 123, 463-469.

Pringle, B., Bishop, C. and Clayton, R. (2009), *Potatoes postharvest.* Available at: *www.cals.wisc.edu/ars/hancock/documents/**PotatoesPostHarvest**.pdf* - Accessed: 10th January, 2015)

Ravi and Aked (1996). Review on tropical roots and tuber crops II. Physiological disorders in fleshly stored roots and tubers. *Critical Reviews in food Science and Nutrition*, 36(7), 711-731

Ray, R.C. and Ravi, V. (2005). Post-harvest spoilage of sweet potato and its control measures. *Critical Review of Food Science and Nutrition* 35, 623-644

Rees, D., van Oirschot, Q.E.A., Amour, R., Rwiza, E., Kapinga, R. and Garey, T. (2003). Cultivar variation in keeping quality of sweet potatoes. *Postharvest Biology and Technology*, 28, 313–325

Rentzsch, S., Podzimska, D., Voegele, A., Imbeck, M., Muller, K., Linkies, A. and Leubner-Metzger, G. (2012). Dose- and tissue-specific interaction of monoterpenes with the gibberellin-mediated release of potato tuber bud dormancy, sprout growth and induction of alpha-amylases and beta-amylases. *Planta,* 235, 137–151

Rossouw, J.A. (2008), *Effect of cytokinnin and gibberellins on potato tuber dormancy.* MSc Thesis, University of Pretoria.

Saltveit, M.E. (1999), Effect of ethylene on quality of fresh fruits and vegetables. *Postharvest Biology and Technology*, 15, 279-292.

Sesay, K., George, J.B. and Kamara, A.A. (1989), Comparative study of different clamp designs for sweet potato storage. Institute of Agricultural Research, Sierra Leone, annual report 1988/89; p. 25

Sonnewald, S. and Sonnewald, U. (2014). Regulation of potato tuber sprouting. *Planta*, 239, 27–38

Suttle J. (2007) Dormancy and sprouting. Advances and perspectives. Elsevier BV, Vreugdenhil D (ed) Potato biology and biotechnology, p. 287–309

Suttle J (2008). Effects of synthetic phenylurea and nitroguanidine cytokinins on dormancy break and sprout growth in Russet Burbank mini tubers. *American Journal of Potato Research*, 85, 121–128.

Thottappily, G. (2009). *The sweet potato.* Springer Science and Business Media *B.V.* ISBN: 978-1-4020-9474-3. Doi: 10-1007/978-1-4020-9475-0

Tortoe,C. Mary Obodai,M. Amoa-Awua,W. (2010). Microbial deterioration of white variety sweet potato (*Ipomoea batatas*) under different storage structures. *International Journal of Plant Biology,* 1, 52-55.

van Oirschot, Q.E.A., Rees, D. and Aked, J. (2003). Sensory characteristics of five sweet potato cultivars and their changes during storage under tropical conditions. *Food Quality Preference*, 14, 673–680.

Zhang, Z.J., Zhang, H.W., Quan, R.D., Wang, X.C. and Huang, R.F. (2009), Transcriptional Regulation of the Ethylene Response Factor LeERF2 in the Expression of Ethylene Biosynthesis Genes Controls Ethylene Production in Tomato and Tobacco. *Plant Physiology*, 150, 365-377

3 THE EFFECTS OF ETHYLENE SUPPLEMENTAL CONTROLLED ATMOSPHERE ON THE PHYSIOLOGY AND BIOCHEMISTRY OF STORED SWEET POTATOES

S. Sowe, S. Landahl, R. Tosetti, M. Anastasiadi and L.A. Terry*

Plant Science Laboratory, Cranfield University, Bedfordshire, MK43 0AL, United Kingdom

*Corresponding author - E-mail: l.a.terry@cranfield.ac.uk ; Tel: +44 (0)1234 750111

Abstract

The effects of CA storage on sweet potato are less reported compared to tuber crops such as potato. Controlled atmosphere storage may serve as an effective additive treatment to ethylene in extending the storage life of sweet potato with minimal adverse effects. There is a paucity of information on the role that CA storage plays in controlling the postharvest physiological deterioration and the biochemical dynamics of sweet potato during storage. The overall objective of this study was to elucidate the efficacy of CA treatments on the physiological and biochemical (non-structural carbohydrates) characteristics of sweet potato during storage. The root samples from a single cultivar (06-52) were placed in 16 L airtight boxes and flushed with gas treatments of 5 kPa CO_2 and 8 kPa O_2 supplemented with and without 0.001 kPa exogenous ethylene and air (21kPa O_2 and 0.003kPa CO_2) stored at 20˚C. At six weeks of storage root samples were transferred across the respective treatments to ascertain application timing effects.

Results showed that ethylene supplemented CA significantly increased respiration rate and weight loss as opposed to continuous CA and the transition across treatments also had a differential effect on the two variables. In as much as CA with and without supplemented ethylene were effective in supressing sprout development during storage, the treatments were characterized with splitting on the surface of the sweet potato. Results also indicated an increased fructose with a corresponding decreased glucose and sucrose accumulation was disproportionately higher during storage compared to the monosaccharides. Continuous CA storage with and without supplemented ethylene had similar effects on the monosaccharides as well as sucrose whereas the treatments accelerated the metabolism of the phenolics over time.

Keywords: *Ipomoea batatas*, sugars, weight loss, respiration rates

3.1 Introduction

Sweet potato (*Ipomoea batatas* L.) is an important nutritious root crop cultivated in more than 100 countries in tropics and sub-tropics including Sierra Leone. Global demand for this agricultural commodity is growing and meeting this requires continuous supply of the crop. However, this is limited by the perishability of the root after harvest as a result of shrivelling, sprouting and disease. Different storage methods such as low temperature storage and continuous exogenous ethylene (Cheema et al. 2013; Amoah et al. 2016) have been used in an attempt to mitigate this problem. However, despite its success in sprout suppression, exogenous ethylene (10 μL L^{-1}) has been characterized with adverse effects such as increased disease incidence and increased sugar contents in sweet potato.

Limiting the biosynthesis of ethylene has been carried out using many strategies including the application of aminoethoxyvinylglycine (AVG) (Cheema 2010), 1-methylcyclopropane (1-MCP) (Amoah and Terry, 2018) and CA storage. The AVG, 1-MCP and CA serve as ethylene binding receptors which actively repress the response of ethylene during its biosynthesis. Controlled atmosphere storage of sweet potato has not been extensively reported compared to other crops of similar physiology such as potato (Khanbari and Thompson, 1996; Ma et al. 2010). This has led to piece meal investigations on the full benefits CA storage has to offer to this important root crop. Cantwell and Suslow (2013) also reported a non-existent commercial use of CA for sweet potato and also a lack of documented information on the response of increased CO_2 levels on the quality of the roots. Due to the similarities in terms of the dormancy and sprouting phenomenon, the roots of sweet potato exhibit similar properties to potato and therefore may also have some similarities in terms of response to various gas compositions. Controlled atmosphere storage has also been reported to be an effective antagonistic agent in terms of minimising the negative effects of exogenous ethylene. Therefore this treatment may serve as an effective alternative and/or an additive treatment to ethylene in extending the storage life of sweet potato with minimal adverse effects.

According to Chang and Kays (1981) weight loss and decay in stored sweet potato roots declined considerably under a CA treatment of 7 kPa O2 plus 2-3 kPa CO2. However, roots stored under less than 7 kPa O2 plus above 10 kPa CO2 developed an unpleasant flavour. Imahori et al. (2007) also reported that a short term exposure of sweet potato to 1 kPa O_2 for seven days at 20˚C has the potential of prolonging the postharvest life of the roots in place of low temperature. Almost a decade earlier Kotecha and Kadam, (1998) had reported that sweet potato held at 2-3 kPa CO_2 and 7 kPa O_2 incurred fewer losses in comparison to those stored in air. The authors further pointed out that an increased level of CO_2 above 10 kPa and an O_2 level below 7 kPa are associated with off flavour whereas O_2 levels between 2.5 and 5 kPa enhanced the accumulation of total sugars.

Storage pests such as insects (e.g. weevils) are generally killed more rapidly by increased CO_2 than they are by the lack of oxygen. However, levels of 40-60 kPa CO_2 and 2-4 kPa O_2 at 25˚C during storage have been reported to cause increased decay and off-flavour in the roots of sweet potato as well as 100% mortality in adult weevils (Delate et al., 1990). Subsequent report by Mitcham et al. (2003) confirms the efficacy of CA storage in the control of insects in fresh horticultural produce.

Khanbari and Thompson (1996) have reported a complete sprout inhibition, low weight loss and maintenance of healthy skin in potato cultivars stored at CA regimes of 9.4 kPa and 6.4 kPa CO_2 and 3.6 kPa O_2 at 5°C for 25 weeks. The authors reported a maintained healthy skin and no indication of sprouting for a further period of 20 weeks when the tubers were stored in air at the same temperature. However despite the benefits of extended postharvest life and physical quality, crisps derived from these stored potato tubers were characterized with dark fry colour which falls short of the required industry standard.

The effects of CA storage with respect to the physiological dynamics and associated biochemical changes in the root of sweet potato are yet to be fully investigated. Hence, the overall objective of this study was to determine the efficacy of CA storage with and without supplemented ethylene at 20˚C, on the physiological (dormancy and sprouting, respiration rates) and biochemical (non-structural carbohydrates, polyphenols and plant growth hormones) properties of sweet potato.

3.2 Materials and methods

3.2.1 Plant material and experimental design

The sweet potato roots (cultivar: 06-52; known as Belle Vue in the USA) used in this study were grown in Senegal, West Africa under ambient conditions (Barfoots of Botley Ltd., West Sussex, UK). The roots were cured for four days at ambient conditions (25 - 30˚C) and subsequently transported to the UK by sea for seven days at 15˚C. Two experiments were conducted in this study: For Experiment 1 (n = 200; storage duration: six weeks), roots were harvested on 10th June, 2015 and then delivered at Cranfield University on 26th June, 2015. For Experiment 2 (n = 400; storage duration: 12 weeks), roots were harvested on 23rd December, 2015, and delivered on 18th January, 2016. The root samples were sorted and divided into two batches: one for baseline sampling and the other placed into 16 L Lock & Lock (HPL890 - 361 × 274 × 212 mm) polypropylene boxes each fitted with inlet and outlet metal connectors, and stored under the following conditions: (1) controlled atmosphere (CA: 8 kPa O_2 and 5 kPa CO_2) ; (2) CA supplemented with 0.001 kPa ethylene and (3) continuous air (21 kPa O_2 and 0.003 kPa CO_2), at 20˚C under normal atmospheric pressure. In Experiment 2, four additional treatments were applied at six weeks as follows: (4) continuous air followed by CA (8 kPa O_2 and 5 kPa CO_2) and vice versa and (5) continuous air followed by ethylene supplemented CA (8 kPa O_2; 5 kPa CO_2 and 0.001kPa) and vice versa. The storage boxes were arranged in a complete randomized design in three replicates (one box per replicate – pseudo-reps: n = 18) and sampling was done at three weeks interval (Experiment 1) and at one, two, three, six, nine and 12 weeks of storage (Experiment 2).

The required gas composition in each box was attained by mixing N_2 at 3.3×10^{-5} m^3s^{-1}, air at 4.0×10^{-5} m^3s^{-1} and CO_2 at 4.3×10^{-6} m^3s^{-1} supplied by the ICA6000 (International Controlled Atmosphere System Ltd., Kent, U.K) through gas distribution manifolds (20 way manifolds; Model No.: 950-20-177, Series 950, HNL Engineering Ltd., Stockton-on-Tees, UK). The supplementation of ethylene was carried out as described by Amoah et al., (2016) with slight modification and the relative humidity (ranged between 85-90%) inside the boxes was measured with wireless data loggers (RD Sens, RFS-TH, Prodisei Technologies S.L., Spain)

3.2.2 Physiological assessments

3.2.2.1 Weight loss, splitting and sprouting evaluation during storage

The weight loss during storage was determined using the method described by Rees et al. (2003) with slight modification. The plastic boxes were weighed empty and then weighed with its contents (i.e. sweet potato samples). The difference in weight was noted which indicated the actual weight of the roots before storage. During storage the boxes together with the contained sweet potato samples were weighed and the difference in weight as a result was then expressed as a percentage of the initial weight of the sweet potatoes.

Splitting on the sweet potatoes as a result of the storage treatments was measured using a Vernier calliper over time. The length, width and depth of the splits were measured and recorded during storage.

Sprout assessment during storage was carried out as described by Amoah et al. (2016) with slight modification. The number of sprouts (only growths greater than 1.5 mm long were considered as sprouts) per root was determined by manual counting on a cumulative basis.

3.2.2.2 Determination of respiration rates during storage

Respiration rates during storage were measured using the Sable Respirometry System (Model 1.3.8 Pro, Sable Systems International, NV, USA) and were expressed in g kg^{-1} s^{-1} as described by Collings et al. (2013) and Alamar et al., (2017) with modifications. Respiration measurements were carried out directly from the 16 L air-tight storage boxes in real time by connecting nalgene® sampling tubes from the outlet valve of each box including the empty reference boxes for each storage treatment, to the sable system using the flow (mL min^{-1}) supplied by the ICA6000 system. An auto-sampling program was set up with a flow multiplexer to sample and sequence (every 19 mins) the evolved gases from the respective storage boxes via water vapour pressure detector (RH-300), a carbon-dioxide detector (CA-10, Firmware version 1.05) and an oxygen detector (FC-10, Firmware version 3.0) to determine relative humidity in the evolved gas, CO_2 and O_2 levels respectively. The measured respiration rates were subsequently analysed and

calculated with respect to the roots' weight using ExpeData software Release 1.3.8 Version.

3.2.3 Biochemical assessments during storage

Sweet potato samples were prepared for biochemical analyses (non-structural carbohydrates phyto-hormones and phenolic compounds) following respiration measurements. Flesh (*ca*. 3 mm thick) and peel (*ca*. 1 mm thick) samples from the sweet potato were manually chopped into cubes of similar sizes, extracted from the proximal (i.e. the stem end of the sweet potato), distal (i.e. the root end of the sweet potato) and middle sections (each section was approximately one-third of the entire length of the sweet potato root), snap frozen in liquid nitrogen and then freeze-dried (Scan Vac, Västerås, Sweden) at -55˚C for 10 days in the dark. Following lyophilisation, the samples were ground into powder and stored at -40˚C for further analyses.

3.2.3.1 Extraction and quantification of non-structural carbohydrates (NSCs)

Non-structural carbohydrates (fructose, glucose and sucrose) were quantified using the method described by Amoah et al., (2016) with slight modifications. Samples (150 mg) of sweet potato powder (n = 252) from the respective treatments were mixed with 3 mL of high-performance liquid chromatograph (HPLC) grade methanol:water extraction solvent (62.5:37.5 v/v) in 7 mL Bijou vials. The mixtures were incubated in a shaking water bath at 55ºC for 15 min (vials were vortexed every five minutes for 20s during the incubation period to prevent layering) and the resulting supernatants subsequently filtered (0.2 µm Cronus, PTFE) and stored at -40˚C for further analysis.

The quantification of individual NSCs was carried out with the HPLC – coupled with Evaporative Light-Scattering Detector (ELSD) (Agilent Technologies Ltd., U.K) after diluting the respective samples with distilled water (sample:water ratio - 1:9). The stationary phase column was a Prevail Carbohydrate ES 5m, GRACE, USA (250 mm by 4.6 mm; Part No. 35101; Serial No. N908718) and the mobile phases comprise two solvents: HPLC grade water (solvent A) and 100% Acetonitrile (solvent B). The mobile phase solvents were mixed in accordance with the gradient program of solvent A: 20 – 50% for 15 mins and 50 – 20% for five minutes (to ascertain a linear relationship

between water and acetonitrile) and a further five minutes post run equilibration time at 20%. An auto sampler injection volume of 20 µL was set up at a column temperature of 40˚C both for the calibration standards and the respective samples at a pump flow rate of 6×10^{-6} m^3s^{-1} using Agilent ChemStation software. Two standard calibration curves were generated in this study as was suggested by Amoah et al., (2016): fructose and glucose (concentration range: 0.0375 - 1.2 kg m^{-3}) and sucrose (concentration range: 0.075 - 2.4 kg m^{-3}) (Sigma-Aldrich Company Ltd., Gillingham Dorset, UK). The concentrations of the respective NSCs in each sample were then calculated against peak areas of standard calibration curves generated from GenStat (VSN International Ltd., UK).

3.2.3.2 Extraction and quantification of polyphenols

The phenolic compounds considered in this study included: chlorogenic acid, Isochlorogenic acids A, B and C, caffeic acid, coumaric acid and ferulic acid (pure standards sourced from Sigma-Aldrich Company Ltd., Dorset, UK). The phenolic compounds were extracted from 100mg of the powdered samples (n = 254) by mixing with 1.5 mL of aqueous methanol: water: HCl (70:29.5:0.5v/v) in 7 mL Bijou vials and the resultant mixture incubated in a shaking water bath at 35˚C for 90 min. The samples were vortexed every 15 minutes for 20s during the incubation period, allowed to cool, filtered into brown vials and stored as explained for NSCs.

Quantification of the phenolic compounds was done using the HPLC with Diode-Array Detection (Agilent Technologies Ltd., UK) as described by Amoah et al., (2016) with slight modifications. Due to the disproportionate amount of the phenolic compounds in the peel of the sweet potato compared to the flesh (*ca*. four times) according to Amoah et al. (2016 two mix standard calibration curves were developed in this study. The standard concentration for the peel ranged from 0.02 – 0.1 kg m^{-3} and that of the flesh ranged from 0.01 – 0.05 kg m^{-3}. The concentrations of the identified phenolic compounds in each sample were then calculated against peak areas of standard calibration curves generated from GenStat (VSN International Ltd., UK). The identification of the phenolic compounds in the sweet potato samples was based on their HPLC retention times against the linear calibration of the pure standards.

3.2.3.3 Extraction and quantification of phyto-hormones

Phytohormones (abscisic acid (ABA) and trans-zeatin rhiboside (trans-ZR)) were extracted from 150 mg of each of freeze-dried powder (n = 84) and weighed into 15 mL falcon tubes. A cold extraction solvent (7 mL) of methanol/water/formic acid mixture (75:20:5 v/v) and a further 30 µL of internal standard mix (d_3-DHZR, d_3-DPA, d_5-ABA-GE, d_3-PA, d_4-ABA) at 1000 µg L^{-1} was added to each of the weighed samples. The respective sample mixes were vortexed for 2 mins at a minimum speed of 2000 rpm, stored at 4˚C for 1 hour in the dark and subsequently vortexed for another 2 minutes at a minimum speed of 2000 rpm. The samples were then centrifuged (Heraeus Labfuge 400R Centrifuge, Thermo Scientific, Fischer, UK) for 15 min at 4500 rpm, at 4˚C and the supernatant filtered through a 0.2 µm PTFE filter. A volume of 500 µL of the extraction solvent was passed through each of the filters to ensure maximum recovery of the extracts. The respective sample extracts were then snap frozen in liquid nitrogen and freeze dried overnight at -110˚C. Following freeze drying, 400 µL of reconstitution solvent (water: acetonitrile: formic acid; 90: 9.9: 0.1 v/v) was added to $9/10^{th}$ of the samples; the remaining $1/10^{th}$ was spiked with 30 µL of d6-ABA solution at 1000 ng mL^{-1}. Both reconstituted spiked and non-spiked samples were vortexed for 2 mins at a minimum speed of 2000 rpm and the supernatants transferred to 2 mL Eppendorf tubes, centrifuged for 2 min at 12000 rpm at 4°C, filtered through 0.2 µm PTFE filter into brown HPLC vials and then stored at – 40˚C until required. Quantification of the individual phytohormones was done using the Ultra-Performance Liquid Chromatograph coupled to Quadruple Time of Flight Mass Spectrometry (6540 UPLC/QToF/LCMS, Agilent Technologies) as described by Ortiz et al. (2015) and Amoah et al. (2016) with slight modifications.

3.2.4 Statistical analyses

Data were analysed using the statistical package STATISTICA (version 12). The General Linear model was used to plot the data for residuals and identify outliers and Analysis of variance (ANOVA) was used to demonstrate the main effects of cultivar, treatment, root section and time and the interactions between them. Biochemical data for the tissues (i.e. flesh and peel) of the sweet potato were analysed separately (i.e. flesh data set separately analysed and then that of the peel). The ANOVA tables showed

the main effects of the storage time, treatments, root section and the interactions among them. Baseline values were nested with values obtained after storage including those after the transition between treatments during the analyses with STATISTICA. The values of the data were grouped as initial (baseline), before transition and after transition in order to establish the actual effect of the transition between treatments during storage on the changing physiology and biochemistry of the sweet potato (see appendix A). Least significant difference values (LSD $P<0.05$) were calculated for mean separation. The ANOVA tables were generated using STATISTICA (version 12) and the means between storage treatments were separated and compared using the Least Significant Difference (LSD) at 95% confidence level. Baseline values were nested with values obtained after storage during the analyses. Means were compared using the least significance difference at 5%. Trans ZR values were transformed to Natural Logs in an effort to get better residuals.

3.3 Results

3.3.1 Respiration rates and weight loss during storage

The respiration rates of the sweet potato during storage ranged from 7.0×10^{-7} g $kg^{-1}s^{-1}$ to 8.5×10^{-5} g $kg^{-1}s^{-1}$ in Experiment 1 whilst the weight loss ranged from 1.5% to 13.4% (Table 3-1). Ethylene supplemented CA significantly lowered the respiration rate by one-fourth whilst increasing the weight loss by 13% compared to the baseline. Also ethylene supplemented CA tripled the weight loss of continuous CA during storage with an associated increase 0.05-fold in respiration rate. However, while exhibiting a similar amount of weight loss, ethylene supplemented CA treatment *c.a.* tripled the respiration rate of continuous air storage over time.

In Experiment 2, the weight loss of the sweet potato during storage under continuous air, continuous CA and continuous ethylene supplemented CA were 7.4%, 5.5% and 15.8% respectively with corresponding respiration rates of 4.5×10^{-5} g $kg^{-1}s^{-1}$, 4.1×10^{-5} g kg^{-1} s^{-1} and 9.1×10^{-5} g kg^{-1} s^{-1} respectively (Figure 3-1). Continuous ethylene supplemented CA doubled the respiration rate of the sweet potato as well as its weight loss compared to air storage. Truncating ethylene supplemented CA decreased the weight loss by half whilst tripling the respiration rate compared to its uninterrupted application. Similarly CA treatment followed by air storage *c.a.* quadrupled the

respiration rate of the sweet potato and concomitantly doubled its weight loss as opposed to continuous CA storage. Continuous CA storage yielded the least weight loss as well as reduced respiration rates over time.

Table 3-1 Weight loss (%) and respiration rates (g kg^{-1} s^{-1}) of sweet potatoes stored in: air (21kPa O_2 and 0.003 kPa CO_2); CA (8 kPa O_2 and 5 kPa CO_2) and CA supplemented with ethylene (0.001 kPa) at 20˚C for a period of six weeks (Experiment 1). Values represent weighted means ± standard error and the l.s.d. represents that of the storage time-treatment interaction effect only. Univariate tests of significance was used for the weight loss and respiration rates (over-parameterised model).

Storage time (weeks)	Treatment	Weight loss (%)	Respiration rates (g $kg^{-1}s^{-1}$)
0	Baseline		8.5E-05 ± 5E-06
3	Air	4.76 ± 1.41	7.3E-05 ± 5E-06
	CA	1.53 ± 0.77	6.3E-05 ± 4E-06
	CA + Ethylene	6.87 ± 0.05	7.7E-05 ± 2E-06
6	Air	13.35 ± 0.94	7.0E-06 ± 1E-06
	CA	4.58 ± 0.86	1.6E-05 ± 2E-06
	CA + Ethylene	13.17 ± 0.84	2.0E-05 ± 2E-06
p-value		0.0052	0.0357
$l.s.d_{(0.05)}$		u.d.	9.9E-06

u.d: undetermined

The p-value ($p<0.05$) represents the probability of weight loss and respiration rates generated by the ANOVA table (see Appendix A) as a result of the interaction between storage time and treatment over time.

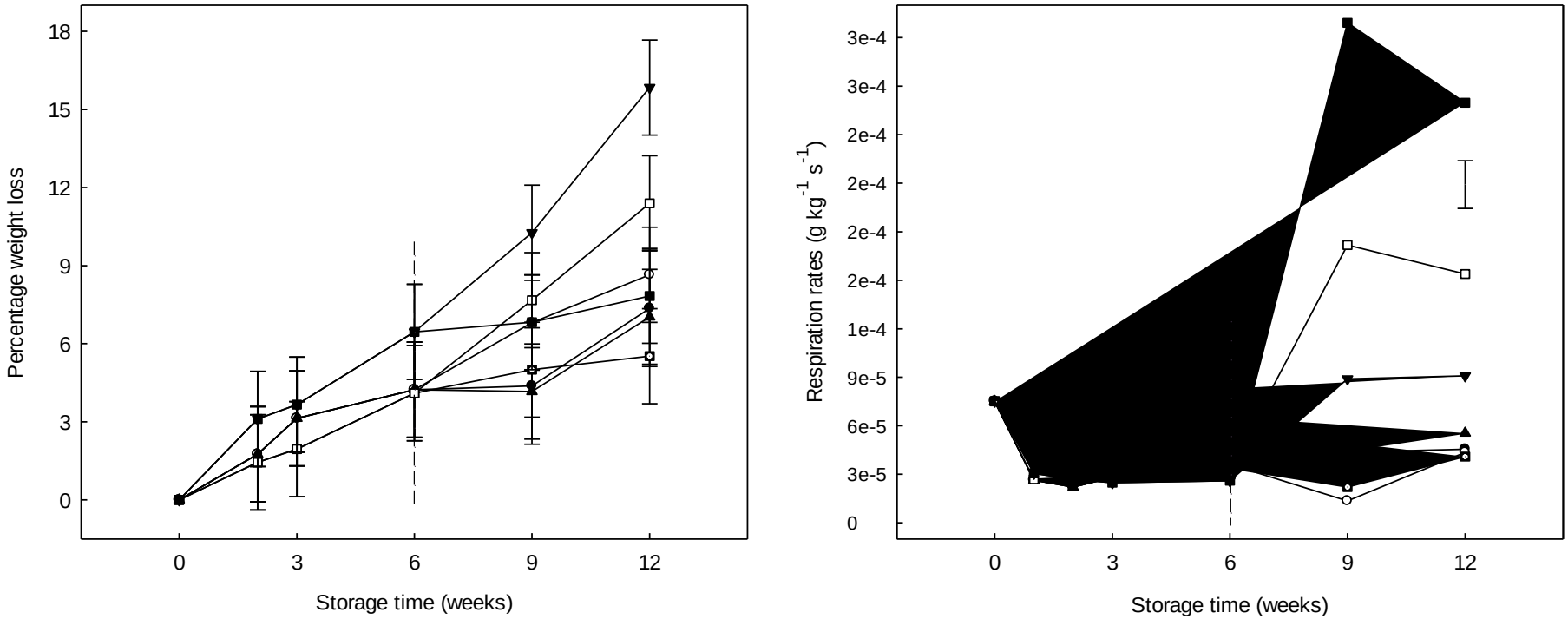


Figure 3-1 Weight loss (%) and respiration rates (g kg^{-1} s^{-1}) of sweet potato (Experiment 2) stored in - (●) air : 21 kPa O_2 and 0.003 kPa CO_2 ; (◘) controlled atmosphere : 8 kPa O_2 and 5 kPa CO_2; (▼) controlled atmosphere supplemented with 0.001 kPa ethylene; (○) air treatment followed by controlled atmosphere; (▲) air treatment followed by controlled atmosphere supplemented with ethylene; (□) controlled atmosphere followed by air; (■) controlled atmosphere supplemented with ethylene followed by air, at 20˚C over 12 weeks. The vertical broken line indicates the time of transition across the respective storage treatments. The $l.s.d._{(0.05)}$ bar shown represent that for storage time-treatment interaction effect during storage. Error bars shown represent the standard errors of the weighted means.

3.3.2 The effects of storage on sprout growth and splitting on sweet potato

Results from this study showed that the storage treatments and the respective transitions significantly influenced the pattern of sprouts growth on the sweet potato (Figure 3-2). Sprout incidence was detected two weeks after storage in continuous air and only after three weeks for those treated with continuous CA and ethylene supplemented CA. Continuous air storage accelerated sprouting by 1.5-fold at the proximal section compared to ethylene supplemented CA whilst doubling the sprout growth of continuous CA storage (Figure 3-4). Meanwhile, ethylene supplemented CA storage followed by air storage suppressed sprouting by 0.7-fold at the proximal section of the sweet potato compared to continuous air storage. Similarly CA treatment followed by air storage and vice versa, inhibited sprout growth by half at the same section compared to air storage. Sprout incidence on the proximal section of the sweet potato *c.a.* doubled that at the distal section over the same time period but the pattern of sprout growth were similar during storage. Sweet potatoes treated with continuous air incurred the greatest incidence of sprouting irrespective of the spatial orientation over time. In contrast, air storage followed by ethylene supplemented CA was the most effective in suppressing sprouting of the sweet potato during storage.

CA to Air **CAE to Air**

Figure 3-2 Sprout growth during storage of sweet potatoes (cultivar: 06-52), in - air: 21 kPa O_2 and 0.003 kPa CO_2; controlled atmosphere (CA: 8 kPa O_2 and 5 kPa CO_2) and CA supplemented with 0.001 kPa ethylene, at 20˚C for a period of 12 weeks. The pictures above shows sprouting in sweet potato as a result of the treatment transition from CA to air and that from CA supplemented with ethylene (CAE) to air during storage.

Splitting (Figure 3-3 below) on the surface of the sweet potato during storage under CA with and without ethylene supplementation was also noticed after nine and six weeks of storage respectively. Those splits provided avenues for sprout development as was observed across the treatment transitions.

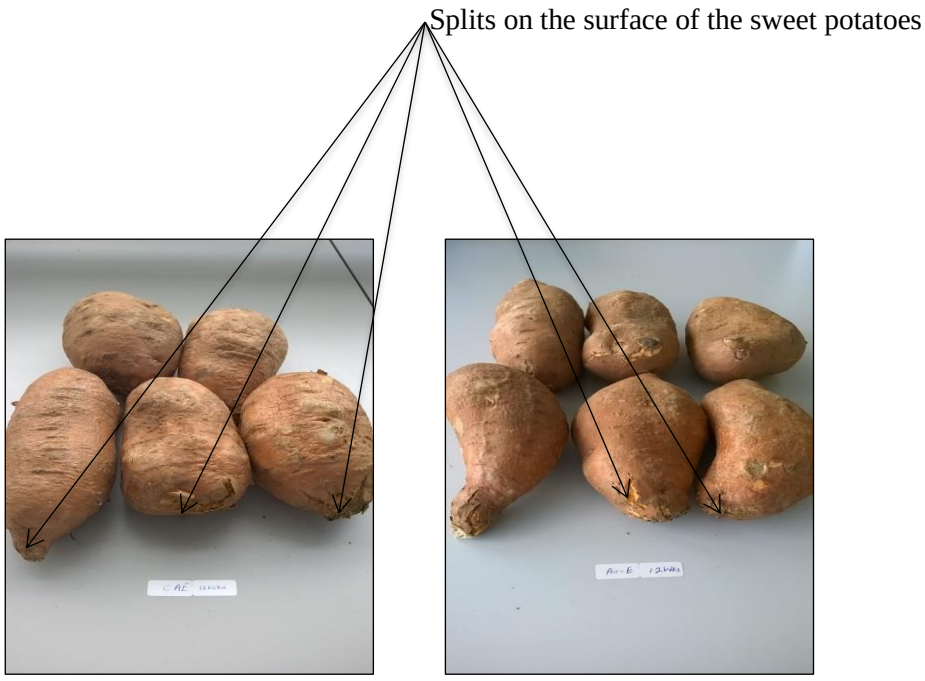


Figure 3-3 The effects of storage treatments on splitting on the surface of sweet potato at 20˚C. The problem of splitting was observed after nine weeks of storage in CA with and without ethylene and also after the transition from air treatment to CA supplemented with ethylene.

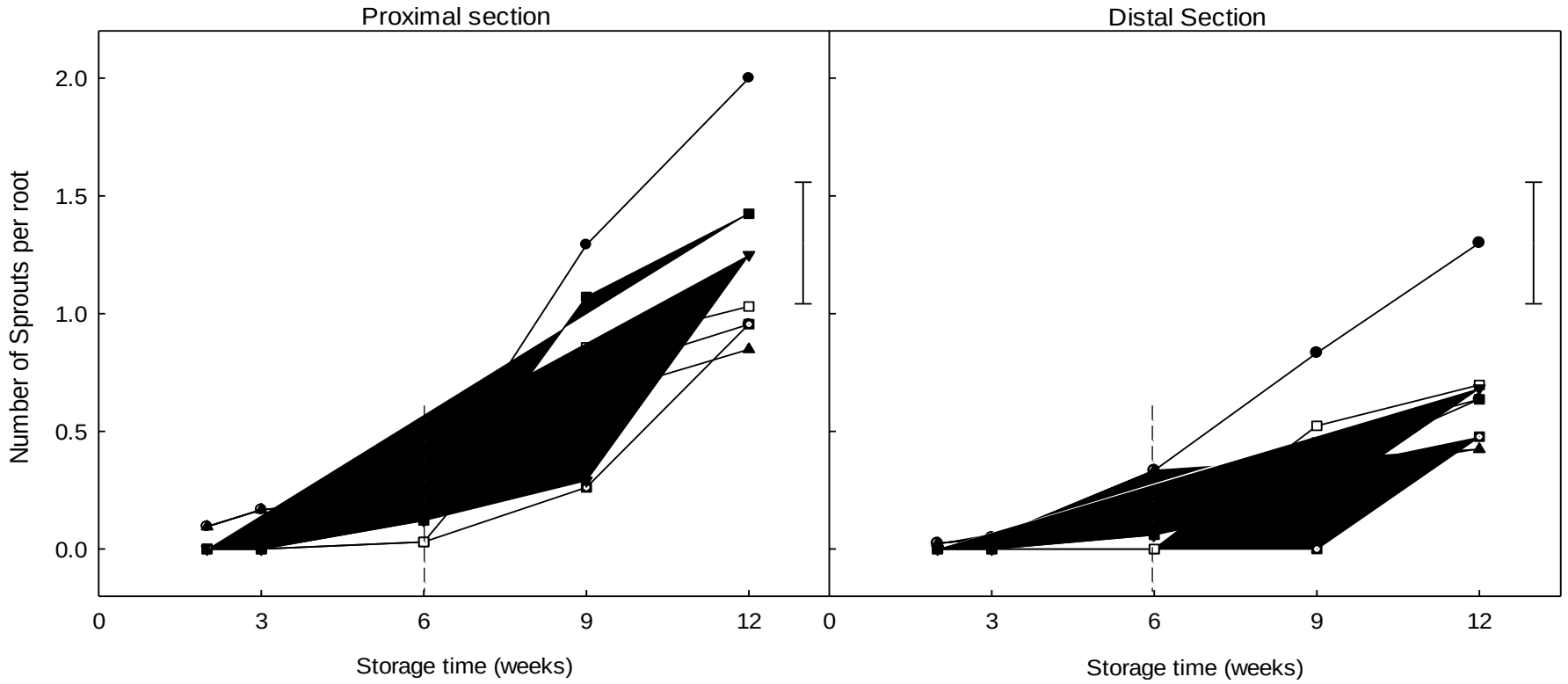


Figure 3-4 The effects of storage treatments -(●) air : 21 kPa O_2 and 0.003 kPa CO_2 ; (◘) controlled atmosphere : 8 kPa O_2 and 5 kPa CO_2; (▼) controlled atmosphere supplemented with 0.001 kPa ethylene; (○) air treatment followed by controlled atmosphere; (▲) air treatment followed by controlled atmosphere supplemented with ethylene; (□) controlled atmosphere followed by air; (■) controlled atmosphere supplemented with ethylene followed by air, on sprout development in sweet potato (cultivar: 06-52), stored at 20˚C for a period of 12 weeks at the proximal and distal sections of the root. The vertical broken line indicates the time of transition across storage treatments. The l.s.d.$_{(0.05)}$ bars shown in the figures above represent that for storage time-treatment interaction effect during storage.

3.3.3 The effects of storage on non- structural carbohydrates (NSCs)

In Experiment 1, there was increased accumulation of fructose in the flesh of the stored sweet potato with a corresponding decline in glucose (Table 3-2). Flesh fructose accumulation at the proximal section was enhanced by 1.4-fold and 1.6-fold during storage in air and CA respectively. However, the monosaccharides exhibited the opposite effect in the peel whereas sucrose (the most abundant sugar) increased significantly in the flesh and peel during storage. Ethylene supplemented CA doubled flesh sucrose accumulation whilst air storage facilitated its accumulation by 1.5-fold over time (Table 3-3).

The accumulation of the reducing sugars in Experiment 2 was similar to Experiment 1 in spite of the spatial difference. Air storage tripled and *c.a.* doubled flesh fructose concentration at the proximal and distal sections respectively. On the other hand flesh glucose was significantly inhibited at the proximal section (0.8-fold) during storage in continuous CA with and without ethylene supplementation compared to continuous air storage (Figure 3-4). Truncating ethylene supplemented CA boosted flesh fructose accumulation by 1.5-fold compared to air storage. The reducing sugars in the peel followed a similar pattern as in the flesh during storage irrespective of storage treatment and transitions (Figure 3-5). However, sucrose accumulation in the sweet potato was inhibited during storage albeit slightly in both the flesh and peel tissues across the different treatments.

Table 3-2 Fructose and glucose concentrations (g kg^{-1} dry weight basis) of sweet potatoes during storage as affected by treatment with: air (21 kPa O_2 and 0.003 kPa CO_2); controlled atmosphere (CA: 8 kPa O_2 and 5 kPa CO_2); CA supplemented with 0.001 kPa ethylene (CA + Ethylene) at 20˚C for a period of six weeks: Experiment 1 (2014-15). Values represent weighted mean ± standard error and the l.s.d. represents the treatment effect only

Storage time (weeks)	Treatment	Fructose (g kg^{-1})				Glucose (g kg^{-1})			
		Flesh		Peel		Flesh		Peel	
		Proximal	Distal	Proximal	Distal	Proximal	Distal	Proximal	Distal
0	Baseline	92.2 ± 8.2	68.3 ± 25.2	26.2 ± 0.5	30.7 ± 4.7	45.1 ± 4.1	34.3 ± 13.7	12.5 ± 0.5	14.7± 1.9
3	Air	99.9 ± 18.1	111.7 ± 6.4	19.6 ± 1.5	18.4 ± 0.6	52.4 ± 13.8	67.8 ± 4.9	17.1 ± 1.8	16.3 ± 1.1
	CA	76.9 ± 2.6	103.4 ± 17.0	22.6 ± 6.4	18.6 ± 0.8	28.7 ± 1.2	56.8 ± 8.6	17.4 ± 3.2	16.8 ± 1.1
	CA + Ethylene	85.3 ± 10.7	116.5 ± 17.5	20.1 ± 1.5	19.5 ± 2.3	35.0 ± 5.3	61.5 ± 7.9	15.0 ± 0.8	15.1 ± 0.9
6	Air	132.9 ± 18.5	140.6 ± 14.1	24.4 ± 8.9	26.2 ± 5.4	41.7 ± 10.1	29.4 ± 7.9	13.6 ± 4.4	18.1 ± 2.6
	CA	144.9 ± 17.9	150.7 ± 18.8	19.1 ± 4.1	25.9 ± 5.5	38.4 ± 8.2	33.9 ± 12.6	14.8 ± 2.8	18.9 ± 3.2
	CA + Ethylene	106.6 ± 7.5	132.5 ± 11.7	22.6 ± 3.8	25.5 ± 10.6	40.8 ± 5.2	28.2 ± 4.3	13.0 ± 1.6	17.9 ± 5.4
p-value		0.0558		0.9153		0.0758		0.4596	
$l.s.d_{(0.05)}$		ns		ns		ns		ns	

ns: non-significant

The p-value ($p<0.05$) represents the probability of monosaccharides metabolism generated by the ANOVA table (see Appendix A) as a result of the interaction between storage time and treatment over the six weeks period.

Table 3-3 Sucrose concentrations (g kg^{-1} dry weight basis) of sweet potato during storage as affected by treatment with: air (21 kPa O_2 and 0.003 kPa CO_2); controlled atmosphere (CA: 8 kPa O_2 and 5 kPa CO_2); CA supplemented with 0.001 kPa ethylene (CA + Ethylene) at 20˚C for a period of six weeks: Experiment 1 (2014-15). Values represent weighted mean ± standard error and the $l.s.d._{(0.05)}$ represents treatment the effect only.

Storage time (weeks)	Treatment	Sucrose (g kg^{-1})			
		Flesh		Peel	
		Proximal	Distal	Proximal	Distal
0	Baseline	107.5 ± 19.9	128.3 ± 10.9	135.2 ± 9.7	159.4 ± 10.0
3	Air	124.6 ± 8.9	115.7 ± 10.2	88.0 ± 2.3	97.0 ± 5.3
	CA	115.4 ± 0.9	172.7 ± 17.4	136.4 ± 23.1	119.1 ± 4.8
	CA + Ethylene	154.4 ± 11.1	179.7 ± 13.7	209.9 ± 8.5	234.1 ± 15.9
6	Air	160.9 ± 24.1	158.5 ± 22.9	138.1 ± 10.5	168.4 ± 19.5
	CA	147.3 ± 18.6	192.5 ± 13.4	160.9 ± 5.5	171.6 ± 22.1
	CA + Ethylene	243.4 ± 2.4	249.2 ± 5.8	237.3 ± 18.6	253.5 ± 22.2
p-value		0.0000		0.0000	
$l.s.d_{(0.05)}$		21.15		20.41	

The p-value ($p<0.05$) represents the probability of sucrose metabolism during storage, generated by the ANOVA table (see Appendix A) as a result of the interaction between storage time and treatment over time.

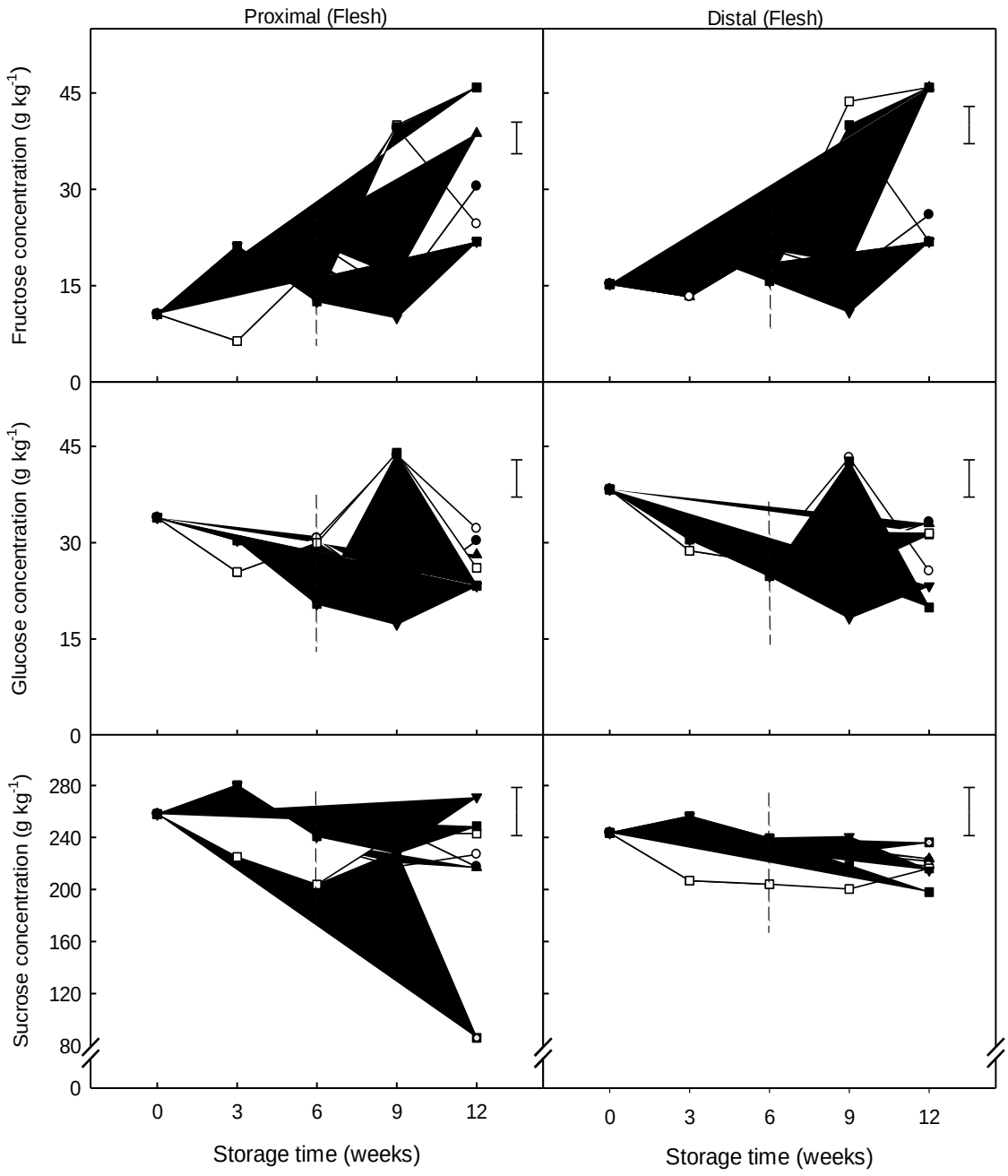


Figure 3-5 The effects of storage in- (●) air : 21 kPa O_2 and 0.003 kPa CO_2 ; (◘) controlled atmosphere : 8 kPa O_2 and 5 kPa CO_2; (▼) controlled atmosphere supplemented with 0.001 kPa ethylene; (○) air treatment followed by controlled atmosphere; (▲) air treatment followed by controlled atmosphere supplemented with ethylene; (□) controlled atmosphere followed by air; (■) controlled atmosphere supplemented with ethylene followed by air, on sugars metabolism in the flesh tissues at the proximal and distal sections of the roots of sweet potatoes, at 20˚C over 12 weeks. The vertical broken line indicates the time of transition across the respective storage treatments. The l.s.d.$_{(0.05)}$ bars shown in the figures above represent that for storage time-treatment interaction effects only during storage.

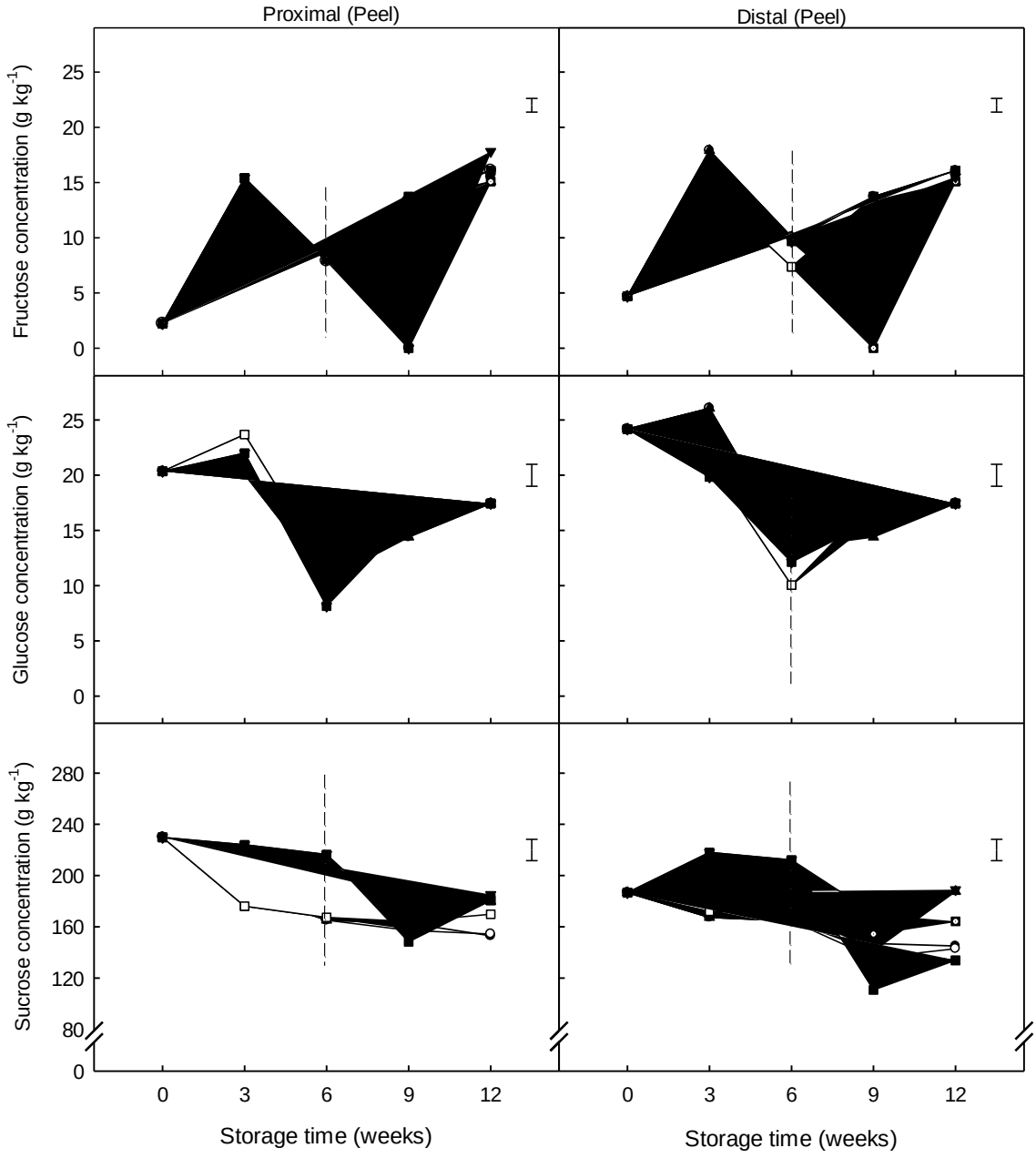


Figure 3-6 The effects of storage in - (●) air : 21 kPa O_2 and 0.003 kPa CO_2 ; (◘) controlled atmosphere : 8 kPa O_2 and 5 kPa CO_2; (▼) controlled atmosphere supplemented with 0.001 kPa ethylene; (○) air treatment followed by controlled atmosphere; (▲) air treatment followed by controlled atmosphere supplemented with ethylene; (□) controlled atmosphere followed by air; (■) controlled atmosphere supplemented with ethylene followed by air, on sugars metabolism in the peel tissues at the proximal and distal sections of the roots of sweet potatoes, at 20˚C over 12 weeks. The vertical broken line indicates the time of transition across the respective storage treatments. The l.s.d.$_{(0.05)}$ bars shown in the figures above represent that for storage time-treatment interactions only.

3.3.4 The effects of CA storage on phenolic compounds and phyto-hormones

The peel concentration of chlorogenic acid and its isomers were exponentially higher than that in the flesh during storage (Experiment 1 and 2). Isochlorogenic acid A largely decreased in the flesh and peel unlike Isochlorogenic acid C during storage. Ethylene supplemented CA significantly raised flesh Isochlorogenic acid A by 1.5-fold at the proximal section with a concomitant decrease of 0.7-fold ($p<0.05$) at the distal section compared to continuous air storage (Table 3-4). Continuous CA storage on the other hand increased Isochlorogenic acid B in the peel by 2.3-fold at the proximal section over time. Furthermore, caffeic and ferulic acids concentrations were doubled and quadrupled respectively at the proximal section during CA storage (Table 3-6). However, treatment transition from ethylene supplemented CA to air storage (Experiment 2) yielded a reciprocating effect on the metabolisms of caffeic and ferulic acids (Figure 3-8).

Flesh chlorogenic acid accumulation was inhibited in Experiment 1 but boosted in Experiment 2 during storage under CA with and without ethylene supplementation. The other studied phenolic compounds in Experiment 2 followed a similar pattern of metabolism to that in Experiment 1. The transitions across the storage treatments (Experiment 2) showed a noticeable increase in flesh Isochlorogenic acid C of the sweet potato. Air storage followed by CA treatment supressed flesh chlorogenic acid accumulation by 0.8-fold compared to uninterrupted CA storage (Figure 3-6). In contrast, Isochlorogenic acid B was boosted by 1.7-fold and *c.a.* tripled at the proximal and distal sections respectively as a result of truncating ethylene supplemented CA as opposed to its continuous application.

The biosynthesis of abscisic acid (ABA) was inhibited considerably in the flesh and peel tissues during storage under ethylene supplemented CA (Table 3-7). Likewise, air storage decreased ABA concentration by half whilst CA storage decreased its concentration to *c.a.* a quarter of the baseline levels irrespective of the root tissue over time. Trans-zeatin riboside (trans-ZR) in the distal flesh doubled during storage under ethylene supplemented CA but its biosynthesis was inhibited by one-third compared to air storage. Also CA storage more than doubled the concentration of trans-ZR compared to air storage (Table 3-8).

Table 3-4 The effects of storage conditions: air (21 kPa O_2 and 0.03 kPa CO_2), controlled atmosphere (CA: 8 kPa O_2 and 5 kPa CO_2) and controlled atmosphere supplemented with 0.001 kPa exogenous ethylene (CA + Ethylene), on chlorogenic acid and Isochlorogenic acid A concentrations (mg kg^{-1} dry weight basis) of sweet potatoes stored at 20˚C over a six weeks period (Experiment one: 2014 - 2015). Values represent weighted mean ± standard error and the l.s.d. represents storage time – treatment interaction effect only

Storage time (weeks)	Treatment	Chlorogenic acid (mg kg^{-1})			
		Flesh		Peel	
		Proximal	Distal	Proximal	Distal
0	Baseline	405.4 ± 13.6	437.2 ± 27.9	2254.3 ± 218.2	2664.8 ± 101.3
3	Air	309.6 ±17.4	305.0 ± 10.3	2075.6 ± 125.9	1844.5 ± 332.1
	CA	406.3 ± 67.4	412.9 ± 29.0	2285.6 ± 95.2	2443.4 ± 296.2
	CA + Ethylene	637.9 ± 58.4	471.0 ± 69.8	2081.4 ± 187.1	2306.8 ± 298.4
6	Air	245.9 ± 40.8	308.9 ± 44.1	2391.6 ± 322.6	2400.2 ± 116.8
	CA	258.5 ± 31.4	246.2 ± 27.8	2658.3 ± 180.3	2620.2 ± 658.1
	CA + Ethylene	365.6 ± 32.0	347.4 ± 60.1	1904.8 ± 209.1	2336.9 ± 217.5
p-value		0.0237		0.4388	
$l.s.d._{(0.05)}$		86.69		ns	
Storage time (weeks	Treatment	Isochlorogenic acid A (mg kg^{-1})			
		Flesh		Peel	
		Proximal	Distal	Proximal	Distal
0	Baseline	433.7 ± 30.0	535.3 ± 41.6	2563.1 ± 165.7	3256.8 ± 150.2
3	Air	178.1 ± 19.9	209.9 ± 20.0	2527.2 ± 217.9	2462.7 ± 506.5
	CA	195.4 ± 71.1	201.8 ± 17.7	2241.1 ± 80.7	2411.5 ± 168.9
	CA + Ethylene	343.9 ± 64.0	225.9 ± 55.2	2179.1 ± 15.4	2368.9 ± 52.7
6	Air	218.9 ± 36.6	316.6 ± 29.9	3294.1 ± 426.8	3463.8 ± 125.5
	CA	182.7 ± 20.8	175.4 ± 17.2	2449.2 ± 357.8	2433.1 ± 542.5
	CA + Ethylene	326.2 ± 36.8	224.1 ± 90.7	1978.9 ± 100.2	1914.5 ± 158.9
p-value		0.2909		0.0146	
$l.s.d._{(0.05)}$		ns		560.34	

The p-value ($p<0.05$) represents the probability of the metabolisms of chlorogenic acid and Isochlorogenic acid A generated by the ANOVA table (see Appendix A) as a result of the interaction between storage time and treatment over time.

Table 3-5 The effects of storage conditions: air (21 kPa O_2 and 0.03 kPa CO_2), controlled atmosphere (CA: 8 kPa O_2 and 5 kPa CO_2) and controlled atmosphere supplemented with 0.001 kPa exogenous ethylene (CA + Ethylene), on Isochlorogenic acids B and C concentrations (mg kg^{-1} dry weight basis) of sweet potatoes stored at 20˚C over a six weeks period (Experiment one: 2014 - 2015). Values represent weighted mean ± standard error and the l.s.d. represents storage time – treatment interaction effect only

Storage time (weeks)	Treatment	Isochlorogenic acid B (mg kg^{-1})			
		Flesh		Peel	
		Proximal	Distal	Proximal	Distal
0	Baseline	104.1 ± 13.9	133.4 ± 33.1	736.7 ± 126.8	1126.9 ± 122.6
3	Air	33.3 ± 2.1	65.4 ± 18.2	591.9 ± 66.3	681.8 ± 182.4
	CA	81.2 ± 30.1	73.5 ± 0.3	696.3 ± 19.9	887.6 ± 153.9
	CA + Ethylene	98.7 ± 37.6	66.8 ± 29.4	740.3 ± 42.3	749.4 ± 73.5
6	Air	102.6 ± 24.9	114.1 ± 22.9	1518.1 ± 285.1	1456.8 ± 71.3
	CA	103.4 ± 14.7	82.6 ± 1.6	1707.9 ± 403.3	1247.8 ± 363.1
	CA + Ethylene	57.4 ± 25.3	46.9 ± 18.3	697.7 ± 27.7	637.4 ± 48.7
p-value		0.0357		0.0032	
$l.s.d._{(0.05)}$		47.35		380.91	
Storage time (weeks)	Treatment	Isochlorogenic acid C (mg kg^{-1})			
		Flesh		Peel	
		Proximal	Distal	Proximal	Distal
0	Baseline	0.0	0.0	163.5 ± 48.7	233.3 ± 33.2
3	Air	0.0	0.0	219.2 ± 23.8	217.5 ± 42.2
	CA	0.0	0.0	189.2 ± 19.4	303.0 ± 58.1
	CA + Ethylene	8.3 ± 0.7	9.8 ± 6.4	295.4 ± 12.8	232.8 ± 41.4
6	Air	5.0 ± 2.6	0.0	555.4 ± 101.9	540.0 ± 38.4
	CA	9.9 ± 1.5	3.6 ± 0.6	566.2 ± 101.6	409.8 ± 58.6
	CA + Ethylene	13.9 ± 5.3	10.3 ± 4.8	345.2 ± 17.9	307.2 ± 19.7
p-value		0.4736		0.0081	
$l.s.d._{(0.05)}$		ns		116.35	

The p-value ($p<0.05$) represents the probability of the metabolisms of Isochlorogenic acids B and C generated by the ANOVA table (see Appendix A) as a result of the interaction between storage time and treatment over time.

Table 3-6 The effects of storage conditions: air (21 kPa O_2 and 0.03 kPa CO_2), controlled atmosphere (CA: 8 kPa O_2 and 5 kPa CO_2) and controlled atmosphere supplemented with 0.001 kPa exogenous ethylene (CA + Ethylene), on caffeic acid and ferulic acid peel concentrations (mg kg^{-1} dry weight basis) of sweet potatoes stored at 20˚C over a six weeks period (Experiment one: 2014 - 2015). Values represent weighted mean ± standard error and the l.s.d. represents treatment effect only

Storage time (weeks)	Treatment	Caffeic acid (mg kg^{-1})		Ferulic acid (mg kg^{-1})	
		Proximal	Distal	Proximal	Distal
0	Baseline	123.8 ± 8.8	165.5 ± 18.4	47.5 ± 12.3	60.4 ± 20.9
3	Air	499.6 ± 56.9	351.7 ± 43.9	28.5 ± 0.9	25.4 ± 3.3
	CA	247.6 ± 22.9	234.7 ± 53.7	24.9 ± 3.3	60.2 ± 37.6
	CA + Ethylene	139.5 ± 23.7	231.2 ± 42.4	113.1 ± 15.4	136.1 ± 17.2
6	Air	274.8 ± 24.0	357.4 ± 53.2	148.5 ± 23.7	131.4 ± 15.9
	CA	250.8 ± 85.1	169.5 ± 36.6	181.3 ± 31.6	136.2 ± 2.1
	CA + Ethylene	129.7 ± 12.4	140.4 ± 15.8	144.7 ± 4.3	138.7 ± 21.1
p-value		0.0000		0.0042	
$l.s.d._{(0.05)}$		58.01		28.02	

The p-value ($p<0.05$) represents the probability of the metabolisms of Caffeic and Ferulic acids generated by the ANOVA table (see Appendix A) as a result of the interaction between storage time and treatment over time.

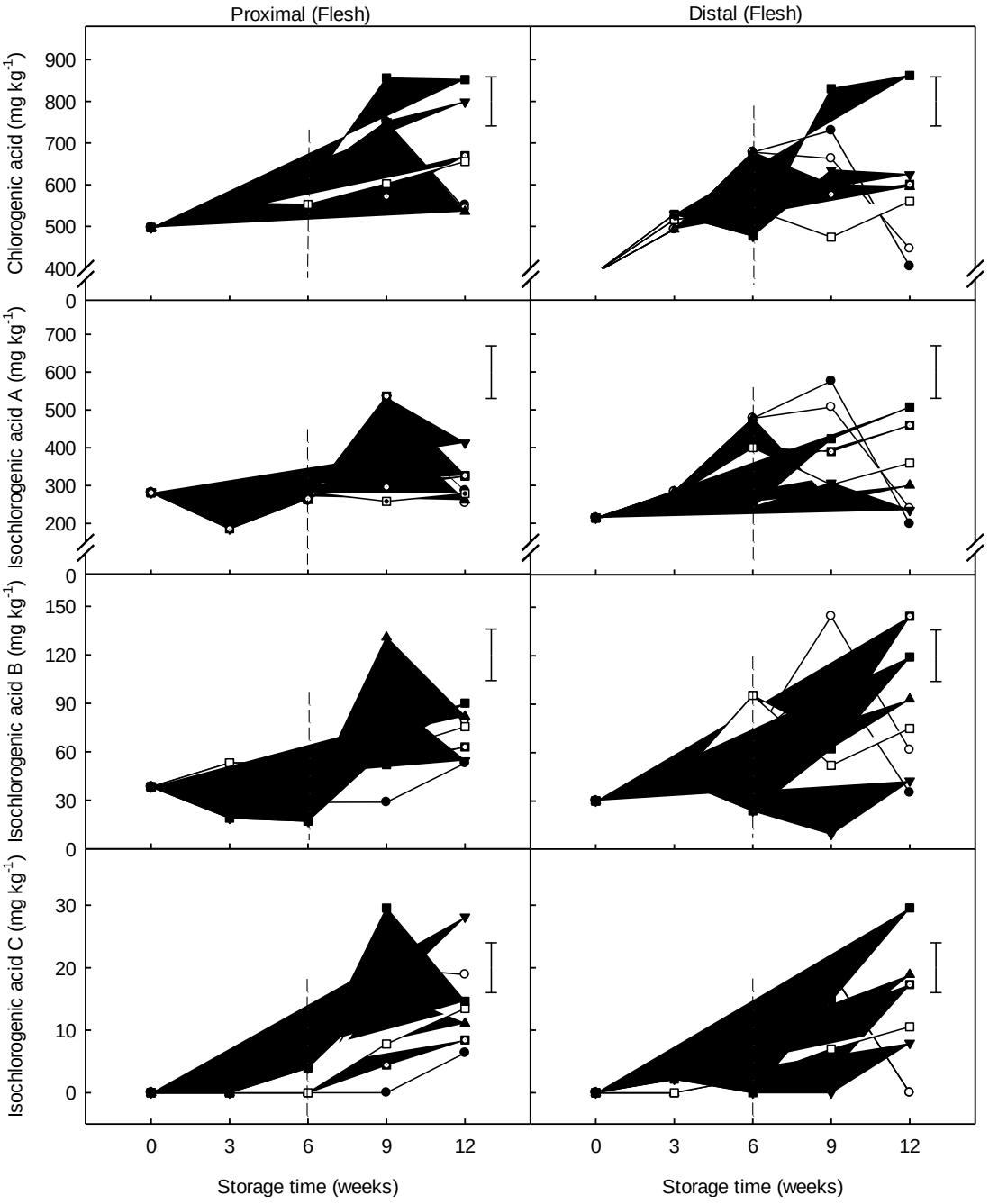


Figure 3-7 The effects of storage in The effects of storage in - (●) air : 21 kPa O_2 and 0.003 kPa CO_2 ; (◘) controlled atmosphere : 8 kPa O_2 and 5 kPa CO_2; (▼) controlled atmosphere supplemented with 0.001 kPa ethylene; (○) air treatment followed by controlled atmosphere; (▲) air treatment followed by controlled atmosphere supplemented with ethylene; (□) controlled atmosphere followed by air; (■) controlled atmosphere supplemented with ethylene followed by air, on polyphenol metabolism in the flesh tissues at the proximal and distal sections of the roots of sweet potatoes, at 20˚C over 12 weeks. The vertical broken line indicates the time of transition across the respective storage treatments. The l.s.d.$_{(0.05)}$ bars shown in the figures above represent that for treatment effects only.

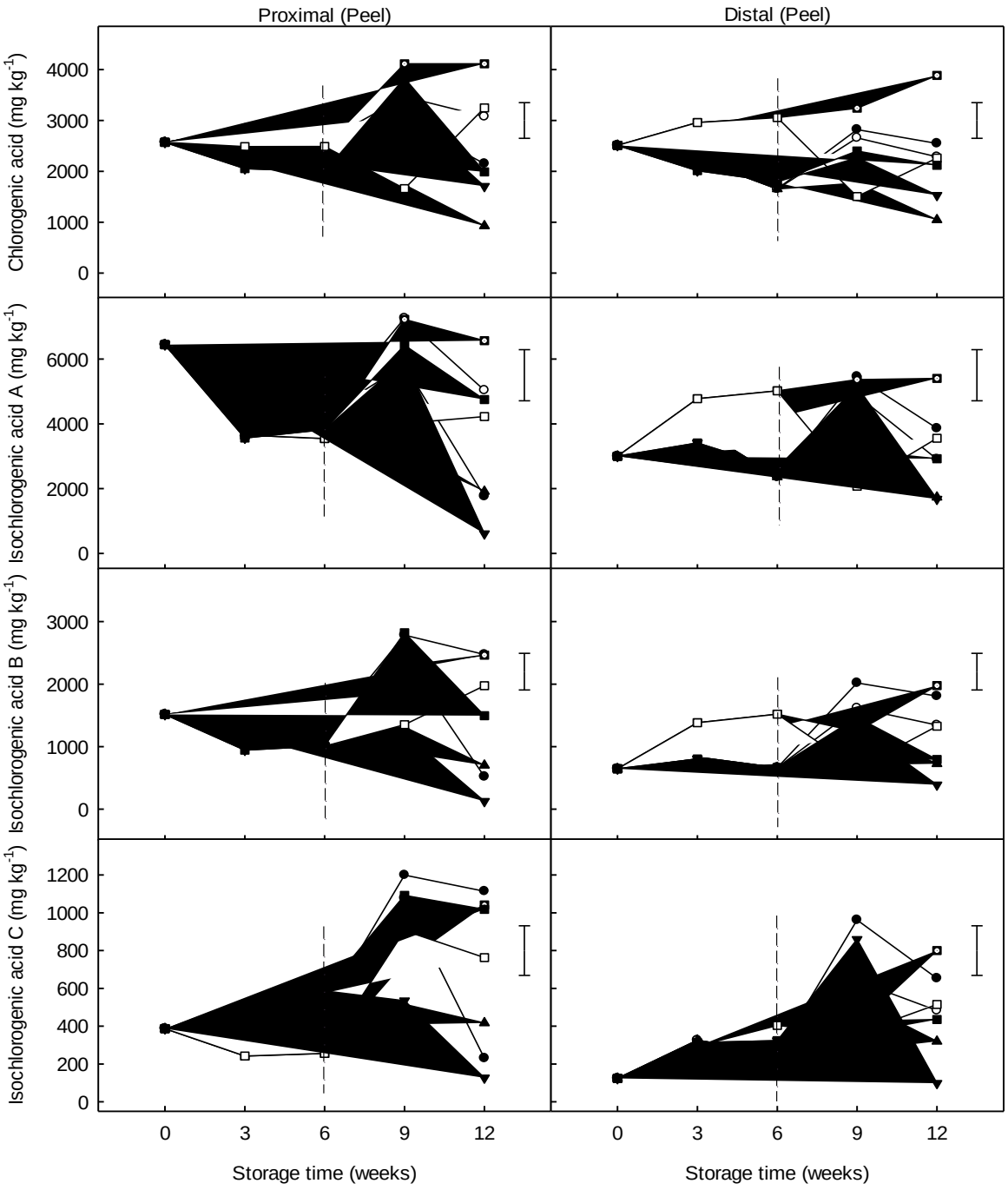


Figure 3-8 The effects of storage in - (●) air : 21 kPa O_2 and 0.003 kPa CO_2 ; (◘) controlled atmosphere : 8 kPa O_2 and 5 kPa CO_2; (▼) controlled atmosphere supplemented with 0.001 kPa ethylene; (○) air treatment followed by controlled atmosphere; (▲) air treatment followed by controlled atmosphere supplemented with ethylene; (□) controlled atmosphere followed by air; (■) controlled atmosphere supplemented with ethylene followed by air, on polyphenol metabolism in the peel tissues at the proximal and distal sections of the roots of sweet potatoes, at 20˚C over 12 weeks. The vertical broken line indicates the time of transition across the respective storage treatments. The l.s.d. $_{(0.05)}$ bars shown in the figures above represent that for storage time-treatment interaction effect only.

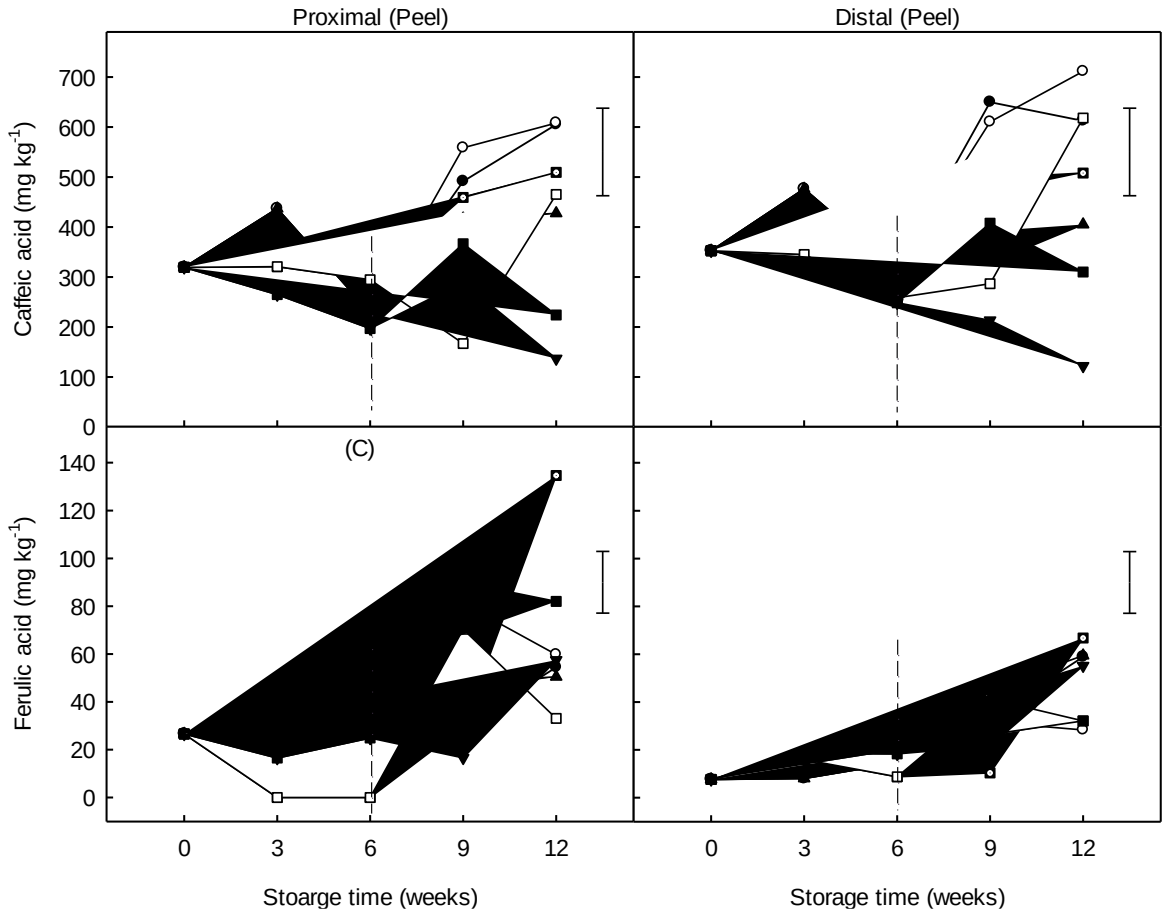


Figure 3-9 The effects of storage in - (●) air : 21 kPa O_2 and 0.003 kPa CO_2 ; (◘) controlled atmosphere : 8 kPa O_2 and 5 kPa CO_2; (▼) controlled atmosphere supplemented with 0.001 kPa ethylene; (○) air treatment followed by controlled atmosphere; (▲) air treatment followed by controlled atmosphere supplemented with ethylene; (□) controlled atmosphere followed by air; (■) controlled atmosphere supplemented with ethylene followed by air, on polyphenol metabolism in the peel tissues at the proximal and distal sections of the roots of sweet potatoes, at 20˚C over 12 weeks. The vertical broken line indicates the time of transition across the respective storage treatments. The l.s.d.$_{(0.05)}$ bars shown in the figures above represent that for storage time-treatment interaction effect only.

Table 3-7 Abscisic acid concentration (ng g^{-1} dry weight basis) of sweet potato roots as affected by storage in air (21 kPa O_2 and 0.003 kPa CO_2), controlled atmosphere (CA: 8 kPa O_2 and 5 kPa CO_2) and CA supplemented with 0.001 kPa ethylene (CA + Ethylene) at a temperature of 20˚C over a period of 12 weeks (Experiment two: 2015 – 2016). Values represent weighted mean ± standard error and the l.s.d. represents treatment effect only

Storage treatment	ABA concentration (ng g^{-1})	
	Flesh	Peel
Baseline	1127.9 ± 149.0	995.9 ± 123.2
Air	533.9 ± 72.9	516.1 ± 57.3
CA	394.8 ± 46.8	381.6 ± 56.0
CA + Ethylene	185.6 ± 47.9	223.6 ± 74.8
p-value	0.0013	0.0244
$l.s.d._{(0.05)}$	174.02	205.46

The p-value ($p<0.05$) represents the probability of ABA biosynthesis on the flesh and peel tissues of the sweet potato generated by the ANOVA table (see Appendix A) as a result of storage treatment over time.

Table 3-8 The effects of storage conditions: air (21 kPa O_2 and 0.03 kPa CO_2), controlled atmosphere (8 kPa O_2 and 5 kPa CO_2) and controlled atmosphere integrated with 0.001 kPa exogenous ethylene, on trans-zeatine riboside (trans-ZR) metabolism of sweet potato stored at 20˚C over a 12 week period. Analyses were done on the flesh tissues across the proximal and distal sections of the roots (Experiment 2: 2015 – 2016). Values represent weighted mean ± standard error and l.s.d.(0.05) represents the storage time *treatment*location interaction effect only.

		trans ZR concentration (ng g^{-1}): Flesh	
Storage time (weeks)	Treatment	Proximal	Distal
0	Baseline	22.4 ± 3.9	6.4 ± 3.0
6	Air	68.7 ± 33.1	63.6 ± 36.8
	CA	92.6 ± 8.9	52.5 ± 24.6
	CA + Ethylene	39.9 ± 16.7	130.1 ± 14.0
12	Air	26.5 ± 3.6	37.9 ± 11.7
	CA	54.1 ± 15.2	107.2 ± 42.7
	CA + Ethylene	36.7 ± 23.4	12.6 ± 12.6
p-value		0.008756	
$l.s.d_{(0.05)}$		63.89	

The p-value ($p<0.05$) represents the probability of trans-ZR biosynthesis at the proximal and distal sections of the sweet potato generated by the ANOVA table (see Appendix A) as a result of the interaction between storage time and treatment over time.

3.4 Discussion

3.4.1 The effects of ethylene supplemented CA storage on changes in weight and respiration rates of sweet potato

Weight loss and sprouting during storage are among the major physiological factors that could significantly reduce the marketability of sweet potato. This study has shown that ethylene supplemented CA significantly increased respiration rate and weight loss (Experiments 1 and 2). The increased metabolic rate as a result of increased respiration creates a stressful environment which triggers greater moisture loss during storage. Controlled atmosphere storage without supplemental ethylene exhibited the least weight losses with associated low respiration rates in both experiments. This suggested mitigation in internal moisture migration to the surface of the sweet potato with an associated reduced metabolic rate due to depleted oxygen and increased CO_2 levels. The transition across storage treatments had a differential effect on weight loss and respiration rates. A positive correlation between respiration rate and weight loss in sweet potato has been reported (Rees et al., 2003). This study showed no correlation between the two factors during storage. Controlled atmosphere storage has also been reported to reduce plant tissue sensitivity to ethylene action (Prange et al., 2006). Although the findings were not specific to sweet potato, this study suggested a link to that phenomenon.

3.4.2 The effects of CA storage on phyto-hormonal dynamics and sprout growth

The storage treatments had a differential effect on sprout incidence on the sweet potato. Ethylene supplementation on sprout control in sweet potato has been reported (Cheema et al., 2013; Amoah et al., 2016). However there is dearth of knowledge on the mechanisms of CA storage with and/or without supplemental ethylene in controlling sprout growth and development in sweet potato. In as much as CA with and without supplemented ethylene were effective in supressing sprout development during storage, the treatments were characterized with splitting on the surface of the sweet potato. Amoah et al., (2016) has also reported splitting in sweet potato during storage under continuous exogenous ethylene (10 µL L^{-1}) supplementation. This adverse effect hampers the postharvest life of the root as much as its marketability. Sweet potatoes

treated with continuous air incurred the greatest sprout growth whereas the transition from air to ethylene supplemented CA was characterized with the least sprout growth. The complete inhibition of sprouting in potato has also been reported during CA storage at 5˚C with extended sprout suppression when stored in air afterwards (Khanbari and Thompson, 1996). The increased level of sprout growth on the stored sweet potato as a result of transitions across storage treatments agrees with earlier reports of accelerated sprouting incidences due to truncating and supplementing ethylene post dormancy break (Amoah et al., 2016). Controlled atmospheres of 60 kPa CO_2, 20 kPa O_2, 20 kPa N_2 and 20 kPa CO_2, 40 kPa O_2, 40 kPa N_2 in combination with ethylene have been reported to decrease the ABA content in potato within 24h (Coleman, 1998) regardless of previous storage temperature.

Sprout growth has also been closely linked with changes in ABA and ethylene in potato during storage (Sonnewald and Sonnewald, 2014; Ordaz-Ortiz et al. 2015; Foukaraki et al., 2016a). The current study indicated that ethylene supplemented CA treatment inhibited ABA biosynthesis considerably compared to air and CA treatments during storage. A similar trend was established with sweet potatoes stored at 25˚C by Amoah et al. (2016) when treated with continuous ethylene and air. The presence of exogenous ethylene in CA may have triggered the rapid depletion of ABA within the sweet potato tissues, which in turn created an enabling environment for dormancy break. Interestingly, despite the fact that ethylene supplemented CA mitigated ABA biosynthesis it was successful in supressing sprouting suggesting a dual role of ethylene in its interaction with ABA and dormancy control. Also sprouting was inhibited with enhanced biosynthesis of trans-ZR as a result of ethylene supplemented CA treatment. The implications of ZR (the major cytokinin in sweet potato) in sprout growth have been reported (Amoah et al., 2016). Increased cytokinin content as well as its sensitivity has been linked to dormancy loss in potato (Suttle, 2004). However the increased trans-ZR content as this study showed did not translate into proliferation of sprouting during storage. This suggested a selective influence of the storage treatments on the biosynthesis of the plant growth hormone. Taking the case of the CA with and without supplemented ethylene treatment, sprout growth was effectively inhibited as opposed to air storage even though trans-ZR accumulation was enhanced in the process. Sugar accumulation has also been linked to sprouting in sweet potato. According to Lin et al.

(2011) sprouting in sweet potato correlated with sugar accumulation and β-amylase activity during storage.

3.4.3 The effects of CA storage with and without supplemented ethylene on changes in NSCs and phenolic compounds of sweet potato

Preliminary studies (Experiment 1) and a repeat of the experiment (Experiment 2) indicated a reciprocating relationship in monosaccharides accumulation (i.e. increased fructose with a corresponding decreased glucose) in the stored sweet potato. Sucrose accumulation which was slightly inhibited in Experiment 2 as opposed to Experiment 1 was disproportionately higher during storage compared to the monosaccharides. On the other hand phenolic metabolism was differentially influenced by the treatments during storage as has been reported earlier by Amoah et al., (2016). Continuous CA storage with and without supplemented ethylene had similar effects on the monosaccharides as well as sucrose whereas the treatments accelerated the metabolism of the phenolics over time. Fructose accumulation agrees with Adu-Kwarteng et al. (2014) who observed a similar pattern in stored sweet potatoes under ambient conditions. The higher content of sucrose could be due to starch hydrolysis triggered by the activation of sucrose phosphate synthase in the root tissues of the sweet potato (Tao et al., 2012; Ponniah et al., 2017). Air storage followed by CA was effective in lowering the NSCs in the flesh of the sweet potato as much as continuous CA. This could be attributed to the reduced metabolism of the NSCs due to depleted O_2 levels. However, although the pattern of accumulation of the NSCs was largely similar, ethylene supplemented CA inhibited all phenolics in the peel as did air storage, whilst continuous CA had the opposite effect.

The disproportionately higher contents of phenolics in the peel than in the flesh of the sweet potato could be related to their facilitation of wound healing and also their likelihood to aiding resistance against pathogens (Duvivier et al., 2010; Jung et al., 2011). Moreover, the exponential increases in the phenolic compounds of sweet potatoes treated with CA with and without ethylene followed by air storage, suggested a correlation with sprout growth. Amoah et al. (2016) have also reported a similar possibility during the break in dormancy of stored sweet potatoes treated with continuous ethylene and air. The increased phenolic content during continuous CA storage in this study also suggested a link to the reduced disease incidence as was

observed in the Experiment 3 (chapter four – section 4.3.1). Aiding natural disease resistance against pathogens by phenolics has also been reported in potato (Pihlanto, 2011).

Increased sweetness in the roots of sweet potato limits its global appeal as a staple food which makes its reduction a necessity if diverse utilization is desired. However, in Taiwan for instance (Huang et al., 2014), sweetness in baked sweet potatoes is desirable whereas in West Africa including Sierra Leone, less sweetness is preferred. Decreased monosaccharides accumulation in sweet potato cultivars Buchbuck and Ibees (Cheema et al., 2013) and Covington (Amoah et al., 2016) have been reported, when treated with ethylene (10 µL L^{-1}) at 25˚C. This study suggested the opposite effect, although ethylene was supplemented with CA.

3.5 Conclusions

In conclusion, results from this study have shown that despite the success in supressing sprouting in sweet potato the additive treatment of CA to ethylene had virtually no positive impact in mitigating the increased metabolism of NSCs. Also the increased weight loss and respiration rates during ethylene supplemented CA storage suggested the non-viability of this treatment in enhancing the postharvest life of the sweet potato. CA must be stressed as well. On the other hand continuous CA showed to be very most effective in sprout suppression as well as reducing weight loss which can enhance the financial gains in terms of marketability of the stored sweet potatoes. However, the problem of splitting after nine weeks of storage limits its longevity in terms of maintaining the quality of the roots over time. Disease incidence during storage was virtually non-existent during continuous CA treatment as opposed to that supplemented with ethylene. The following chapter discusses in detail on specific diseases identified and the associated fungi and the potential mycotoxins that may be produced as a result the studied treatments.

3.6 Acknowledgements

My sincere thanks and appreciation go to the Commonwealth scholarship commission for their support in providing me the opportunity to have this great experience. I would also like to thank members of the plant science group for their technical assistance.

3.7 References

Adu-Kwarteng, E., Sakyi-Dawson, E. O., Ayernor, G. S., Truong, V.-D., Shih, F. F. and Daigle, K. (2014). Variability of Sugars in Staple-Type Sweet Potato (Ipomoea batatas) Cultivars: The Effects of Harvest Time and Storage. *International Journal of Food Properties*, 17(2), 410–420.

Alamar, M. C., Collings, E., Cools, K. and Terry, L. A. (2017). Impact of controlled atmosphere scheduling on strawberry and imported avocado fruit. *Postharvest Biology and Technology*, 134, 76–86.

Amoah, R. S., Landahl, S., and Terry, L. A. (2016). The timing of exogenous ethylene supplementation differentially affects stored sweet potato roots. *Postharvest Biology and Technology*, 120, 92–102.

Amoah, R.S. and Terry, L.A. (2018). 1-Methylcyclopropene (1-MCP) effects on natural disease resistance in stored sweet potato. *Journal of the Science of Food and Agriculture*, in Press

Chang, L. A. and S. J. Kays. (1981). Effects of low oxygen storage on sweet potato roots. *Journal of American Society of Horticultural Science*, 106, 481–483.

Cheema, M. U. A., Rees, D., Colgan, R. J., Taylor, M. and Westby, A. (2013). The effects of ethylene, 1-MCP and AVG on sprouting in sweet potato roots. *Postharvest Biology and Technology*, 85, 89–93.

Collings, E., García Cas, J.A., Ordaz Ortiz, J.J., Terry, L.A. (2013). A new real-time automated method for measuring in-situ respiration rates of fresh produce. *Acta Horticulturae*, 1012, 1151–1157.

Duvivier, P., Hsieh, P.C., Lai, P.Y. and Charles, A.L. (2010). Retention of phenolics, carotenoids, and antioxidant activity in the Taiwanese sweet potato cv. Tainong 66 subjected to different drying conditions. *African Journal of Food, Agriculture, Nutrition and Development*, 11(10), 4413 – 4429.

Foukaraki, G., Cools, K. and Terry, L. A. (2016a). Differential effect of ethylene supplementation and inhibition on abscisic acid metabolism of potato (*Solanum*

tuberosum, L.) tubers during storage, *Postharvest Biology and Technology*, 112, 87–94.

Foukaraki, G., Cools, K., Chope, G. A. and Terry, L. A. (2016b). Impact of ethylene and 1-MCP on sprouting and sugar accumulation in stored potatoes. *Postharvest Biology and Technology*, 114, 95–103.

Huang, C.-L., Liao, W. C., Chan, C.-F. and Lai, Y.-C. (2014). Storage performance of Taiwanese sweet potato cultivars. *Journal of Food Science and Technology*, *51*(12). 4019 – 4025.

Imahori, Y., Kishioka, I., Uemura, K., Makita, E., Fujiwara, H., Nishiyama, Y. and Chachin, K. (2007). Effects of Short-term Exposure to Low Oxygen Atmospheres on Physiological Responses of Sweet potato Roots. *Journal of the Japanese Society for Horticultural Science*, 76(3), 258–265.

Jung, J.-K., Lee, S.U., Kozukue, N., Levin, C. E. and Friedman, M. (2011). Distribution of phenolic compounds and antioxidative activities in parts of sweet potato (*Ipomoea batatas* L.) plants and in home processed roots. *Journal of Food Composition and Analysis*, 24(1), 29–37.

Khanbari, O.S. and Thompson, A.K. (1996), Effect of controlled atmosphere, temperature and cultivar on sprouting and processing quality of stored potatoes; *Potato Research*, 39, 523-531.

Ma, Y., Wang, Q., Gyunghoon Hong, G. and Cantwell, M. (2010). Reassessment of treatments to retard browning of fresh-cut Russet potato with emphasis on controlled atmospheres and low concentrations of bisulphite. *International Journal of Food Science and Technology*, 45, 1486–1494.

Ordaz-ortiz, J., Foukaraki, S., and Terry, L. A. (2015). Assessing temporal flux of plant hormones in stored processing potatoes using high definition accurate mass spectrometry. *Horticultural Research*, 2, 1–8.

Pihlanto, A. (2011). 'Potato and Other Root Crops' in Terry, L.A. (ed) *Health-promoting Properties of Fruits and Vegetables*. CABI, Wallingford, p. 218-233

Ponniah, S. K., Thimmapuram, J., Bhide, K., Kalavacharla, V. and Manoharan, M. (2017). Comparative analysis of the root transcriptomes of cultivated sweet potato (*Ipomoea batatas*, L.) and its wild ancestor (*Ipomoea trifida*, G.). *BMC Plant Biology*, 17(1), 1 – 14.

Prange, R.K., Delong, T.A., Daniels-Lake, B.J. and Harrison, P.A. (2006). Controlled Atmosphere related disorders of fruits and vegetables. *Stewart Postharvest Review*; 5(7), 1-11.

Rees, D., van Oirschot, Q. E. ., Amour, R., Rwiza, E., Kapinga, R., and Carey, T. (2003). Cultivar variation in keeping quality of sweet potatoes. *Postharvest Biology and Technology*, 28(2), 313–325.

Sonnewald, S. and Sonnewald, U. (2014). Regulation of potato tuber sprouting, *Planta*, 239, 27–38

Suttle, J.C. (2004). Physiological regulation of potato tuber dormancy, *American Journal of Potato Research*, 81, 253–262.

Tao, X., Gu, Y.H., Wang, H.Y., Zheng, W., Li, X., Zhao, C.W. and Zhang,Y.-Z.(2012). Digital gene expression analysis based on integrated de novo transcriptome assembly of sweet potato (*Ipomoea batatas*, L.). PLoS *ONE*, 7(4), 1–14.

4 FUNGAL GROWTH AND AFLATOXIN CONTAMINATION ON SWEET POTATO STORED UNDER CONTROLLED ATMOSPHERE CONDITIONS

S. Sowe[a], A. Medina[b], R. Tosetti[a], S. Landahl[a] and L.A. Terry[a*]

[a]Plant Science Laboratory and [b]Applied Mycology Group, Cranfield University, Bedfordshire, MK43 0AL, United Kingdom

*Corresponding author - E-mail: l.a.terry@cranfield.ac.uk ; Tel: +44 (0)1234 750111

Abstract

The control of fungal development in sweet potato during storage is important to minimize disease incidence and also the potential of mycotoxin production, which could be hazardous to human and animal health. This study evaluated fungal disease dynamics and potential associated aflatoxins on sweet potato (cultivar; 06-52) during storage under continuous CA (8 kPa O_2 and 5 kPa CO_2), Air (0.003 kPa CO_2 and 21 kPa O_2), and CA supplemented with 0.001 kPa ethylene, over a period of 12 weeks at 20˚C. Sampling was done using 35g of extracted tissue from the proximal, middle and distal parts of the root and then mechanically mashed with 75mL of sterile water. Serial dilution plating in Potato Dextrose agar (PDA) and Dichloran-Glycerol Agar base (DG 18) was used to evaluate fungal growth and isolated fungal spores were sub-cultured in Yeast Extract Sucrose agar (YES) and subsequently analysed for mycotoxins using a HPLC system (Agilent, UK). Results showed that continuous CA was effective in controlling the growth of *Penicillium* unlike that of *Fusarium*. Disease incidence (mainly *Rhizopus* soft rot and *Fusarium* surface rot) was predominant at the proximal and distal sections during storage under ethylene supplemented CA and air storage. Among the aflatoxins studied, aflatoxin G1 was the most predominantly produced and that continuous CA treatment was very effective in controlling the potential contamination of the studied aflatoxins during storage. Despite the fact that in-vitro analyses was not done, the results could be useful in designing effective control strategies for aflatoxins contamination, hence minimising the health risks associated with fungal activity during storage. The biochemical basis surrounding spatial influence

on fungal development is not fully explained in this report which warrants further investigation.

Keywords: *Ipomoea batatas*, disease, *Fusarium*, *Penicillium*, *Aspergillus*, ethylene

4.1 Introduction

The roots of sweet potato are susceptible to post-harvest deterioration which may occur mainly as a result of microbial activity and disease (Pedreschi et al., 2013) during storage. Fungi are one of the major microbial organisms associated with disease of stored sweet potatoes. Diseases such as: surface rot caused by *Fusarium oxysporum*, root rot caused by *Fusarium solani* and soft rot caused by *Rhizopus stolonifer* (Kays and Kays, 1998; Cantwell and Suslow, 2013), have been reported. The degree of susceptibility of sweet potato to disease occurrence has been shown to be dependent on the cultivar and the storage environment (Holmes and Stange, 2002; Rees et al. 2003). In addition to cultivar dependence, Lewthwaite et al. (2013) also pointed out that disease susceptibility is further exacerbated by piercing wounds on the root surface.

In an attempt to address the problem of disease incidence on sweet potato after harvest, several storage technologies including Low temperature storage, Irradiation, exogenous ethylene, etc. has been used. Fumigation of sweet potato with exogenous ethylene (10 $\mu L\ L^{-1}$) is gradually emerging as an effective means of supressing sprouting during storage. However, its application has been associated with increased disease incidence as a result of microbial activity, mainly fungi (Amoah et al., 2016). Some of the fungi associated with disease incidence on the sweet potatoes could be mycotoxigenic leading to potential fatalities in animals as wells as humans (e.g. liver cancer caused by Aflatoxins). Fungal species such as *Aspergillus* and *Fusarium* can infect the sweet potatoes pre-harvest and mycotoxin contamination can increase if storage conditions are poorly managed. According to Chulze (2010), drying after can minimise or prevent mycotoxin contamination in maize immediately after harvest. The author further pointed out that the critical water content for safe storage corresponds to a water activity (a_w) of about 0.7

Controlled atmosphere storage has also been used to control insect development (mainly weevils) during storage of sweet potato (Delate and Brecht, 1989; Delate et al., 1990).

The control of fungal growth and mycotoxin (e.g. Aflatoxins) contamination in stored commodities using CA has been reported as possible strategies (Chulze, 2010). Additionally, lower levels of oxygen and elevated CO_2 could supress fungal development thereby consolidating the positive aspects of ethylene in sprout control. Also the incidence of disease during storage largely depends on the pathogen-storage treatment interaction. So by creating a hostile environment for fungal spores to grow and develop, decay can be minimized as well as the potential for aflatoxin contamination to arise.

The study of fungal diseases in stored sweet potato is important because they affect the aesthetic quality, storage life and nutritional value of the roots. Also the potential of these pathogens to induce the production of mycotoxins on the root which may be harmful to humans and animals needs to be highlighted. Aflatoxins are potent liver toxins and carcinogens. Preharvest aflatoxin contamination is associated with drought and high temperatures during grain fill. Postharvest aflatoxin contamination can develop when grain is improperly managed through the drying and storage process.

To date there has been little or no available information on fungal growth and its potential mycotoxins production on sweet potato during storage in CA with and without supplemental ethylene. Therefore, the aim of this study was to identify the specific fungi associated with disease occurrence and the associated potential mycotoxins in sweet potato and to also determine the underlying cause of spatial difference during storage under CA with and without supplemental ethylene.

4.2 Materials and methods

4.2.1 Plant material and experimental design

The sweet potato roots (*Ipomoea batatas* L., cultivar: 06-52; known as Belle Vue in the USA) were harvested on 23rd December, 2015 and delivered at Cranfield University on 2nd February, 2016 as explained in chapter three section 3.2.1. The sweet potatoes (n = 200) were sorted and stored for 12 weeks at 20˚C as described under the following conditions: (1) continuous CA (5 kPa CO_2 and 8 kPa O_2); (2) continuous CA supplemented with 0.001 kPa ethylene and (3) continuous air (0.003 kPa CO_2 and 21 kPa O_2). The storage boxes were arranged in a complete randomized design in three

replicates (one box per replicate – pseudo-reps: n = 14) and sampling was done at six weeks interval.

4.2.2 Culture media preparation

Two culture media: Dichloran-Glycerol Agar base (DG 18) and Potato Dextrose Agar base (PDA: Oxoid Ltd, Hampshire, UK) mixed with chloramphenicol (0.05g/L) (for the selective cultivation of fungi from mixed samples.), were used to determine the potential fungal load in the sweet potato samples. The prepared agar media were then autoclaved at 126˚C (Model: Classic Prestige Medical, Meadowrose Scientific Ltd., Oxon, UK) for *ca.* 55 mins, allowed to slightly cool for *ca*. 10 mins, poured into circular plastic petri dishes (Thermo Fisher Scientific Inc., UK) and then left for 20 mins to solidify on cooling in a laminar flow cabinet. After cooling the plates were stacked and placed on a work bench at ambient temperature for the next two days to ascertain non-contamination, before being used. A total of 54 agar plates were prepared for the baseline sampling and for the stored samples a total of 324 agar plates were prepared.

4.2.3 Disease incidence and estimation of fungal populations during storage

Disease incidence (%) of the sweet potatoes during storage was determined cumulatively as described by Amoah et al. (2016) and Alamar et al. (2017) with slight modifications. Diseased roots were identified and manually counted on a cumulative basis until the end of storage and the number of infected roots expressed as a percentage of the initial number of stored roots.

For the estimation of fungal population, a total of nine roots (for baseline sampling) and a total of 54 roots were randomly selected from the sweet potato samples at six and 12 weeks of storage respectively. Root tissues (35g) from the distal, middle and proximal sections were extracted using a sterilized stainless steel scalpel in the laminar flow cabinet. The extracted tissues were crushed into tiny pieces with a sterile blender, mixed with 75 mL of sterilized saline solution (9 g L^{-1} NaCl solution) in clean self-sealed plastic bags (9" × 12.75") and then mechanically mixed into a fine paste for 10 mins using a stomacher (Stomacher, Lab-Blender 400). A one millilitre aliquot from the respective pastes was mixed with 9 mL sterile saline solution (1:10) in three serial dilutions. An aliquot of 100 µL from each dilution was spread with a glass rod on each agar plate in

three replicates and then incubated at 25˚C for seven days in the dark. Fungal populations were subsequently estimated by manual counting and then expressed in colony forming units per milligram (cfu mg^{-1}). The same procedure was repeated for the stored samples across the three storage treatments.

4.2.4 Isolation and identification of fungal species

Individual fungal spores from among the cultured populations in PDA and DG-18 were isolated and sub-cultured in Malt Extract Agar (MEA: Oxoid Ltd, Hampshire, UK) plates and incubated at 25˚C in the dark for seven days. Following incubation, the fungal spores were identified with the aid of a microscope and the pictures that emerged were then compared with that shown in the fungal text book (Samson et al., CBS Laboratory Manual Series). The identified fungal species were then further sub-cultured in Yeast Extract Sucrose Agar {YES: constituted from 20 g L^{-1} of yeast extract (Oxoid Ltd, Hampshire, England), 150 g/L of sucrose (Fisher Scientific, Loughborough, UK) and 15 g L^{-1} agar (Sigma- Aldrich, Co., Steinheim, Germany)} for the *Penicillium* and *Aspergillus* species and in PDA for the *Fusarium* species. The fungal isolates were then incubated for 10 days at 25˚C in the dark for aflatoxin extraction and analyses.

4.2.5 Extraction and analysis for potential production of aflatoxin

Identified *Aspergillus* isolates were analysed for aflatoxin production as described by the AOAC method (2000) before and after storage. Eight 6 mm plugs of fungal biomass were extracted from each agar plate with a sterile core borer and placed in 2 mL Eppendorf tubes in duplicates. Chloroform (1 mL) was then added to each loaded Eppendorf tube and mixed mechanically by shaking for 1 h at 150 rpm in a horizontal orientation. Subsequently, 800 µL of the supernatant from each of the mixes was transferred to a new - 2 mL Eppendorf tube and left open overnight to evaporate. Evaporation of the supernatant was followed by derivatization of the samples as described by Abdel-Hadi et al., (2012) and Astoreca et al., (2014) with modifications. Hexane (200 µL – HPLC grade) and 50 µL Trifluoroacetic acid (TFA) were added to the residue in the 2mL Eppendorf tubes, vortexed for 30 s and then left for 5 mins in the laminar flow cabinet. After that, 950 µL of water : acetonitrile solvent (9:1) was further added to each mix, vortexed for 30 s and left for 10 mins for separation of the layers. After separation, the upper layer in each Eppendorf tube was discarded and the

remaining extract filtered (13 mm, 0.22 µm nylon filters - Kinesis Scientific Experts, Cambridgeshire, UK) into amber vials.

Analyses for aflatoxins were carried out using a HPLC system (Agilent Technologies, Berks., UK) coupled with a 470 fluorescence detector (FLD). The stationary phase was an Agilent Phenomenex Luna ODS2-C18 column (4.6 mm 150 mm, 5µm particle size) and the mobile phases were 30 % methanol, 60 % HPLC grade water and 10% acetonitrile. An auto-sampler injection volume of 20 µL at a flow rate of 1 mL min^{-1} was set up at a run time of 25 mins using a gradient program controlled by Agilent ChemStation software. Identification and quantification of the aflatoxins were based on correlation of their retention times with the linear calibrated standards (Romer Lab, Austria; mycotoxin mix: 2.01 µg mL^{-1} Aflatoxin B1; 2.03 µg mL^{-1} Aflatoxin G1; 0.503 µg mL^{-1} Aflatoxin B2 and 0.504 µg mL^{-1} Aflatoxin G2; mix standards range: 0.5 - 400 ng mL^{-1}) (Abdel-Hadi et al., 2012).

4.2.6 Statistical analyses

Data were analysed using the statistical package STATISTICA (version 12). The General Linear model was used to plot the data for residuals and identify outliers before subjecting to ANOVA. Analysis of variance (ANOVA) tables were generated and the means between storage treatments were separated using the Least Significant Difference (LSD) at 95% confidence level. The ANOVA tables showed the main effects of the storage time, treatments, root section and the interactions among them on potential fungal development and potential production of Aflatoxins from the sweet potatoes. Baseline values were nested with values obtained after storage during the analyses (see appendix B).

4.3 Results

4.3.1 Fungal populations and disease incidence during storage

Results showed that sweet potatoes treated with continuous CA were characterised with significantly less disease incidence compared to continuous air and ethylene supplemented CA treatments over time (Figure 4-1). Also disease incidence at the proximal section of the root doubled ($p<0.05$) that at the distal section whilst the middle section had the least level of decay during storage in air and continuous CA

respectively. Soft rot caused by *Rhizopus stolonifer* and surface rot caused by *Fusarium oxysporum* were predominant at the proximal and distal sections of the sweet potato. Ethylene supplemented CA and air stored roots exhibited *c.a.* equivalent levels of decay over time.

The growth pattern in both PDA and DG-18 media cultures showed that the interactive effect of storage time and treatment significantly influenced fungal population (cfu mg^{-1}) as shown in Table 4-1 and Figure 4-2 below. The growth of fungi during ethylene supplemented CA tripled that of air storage in PDA culture whilst a six-fold rise in growth was recorded when cultured in DG-18. On the other hand, continuous CA storage supressed fungal growth by half compared to air storage over time irrespective of the media culture used.

This study also showed that the proximal section of the sweet potato incurred the greatest increase in fungal development compared to the middle and distal sections during storage. Ethylene supplemented CA storage increased the growth of fungi by six-fold at the proximal section compared to air storage in DG-18 whereas it doubled that of the air stored sweet potato in PDA (Figure 4-2). However, ethylene supplemented CA and air storage exhibited equivalent increases in fungal growths at the distal section irrespective of the media culture used. Sweet potatoes stored under continuous CA incurred the least growth in fungi.

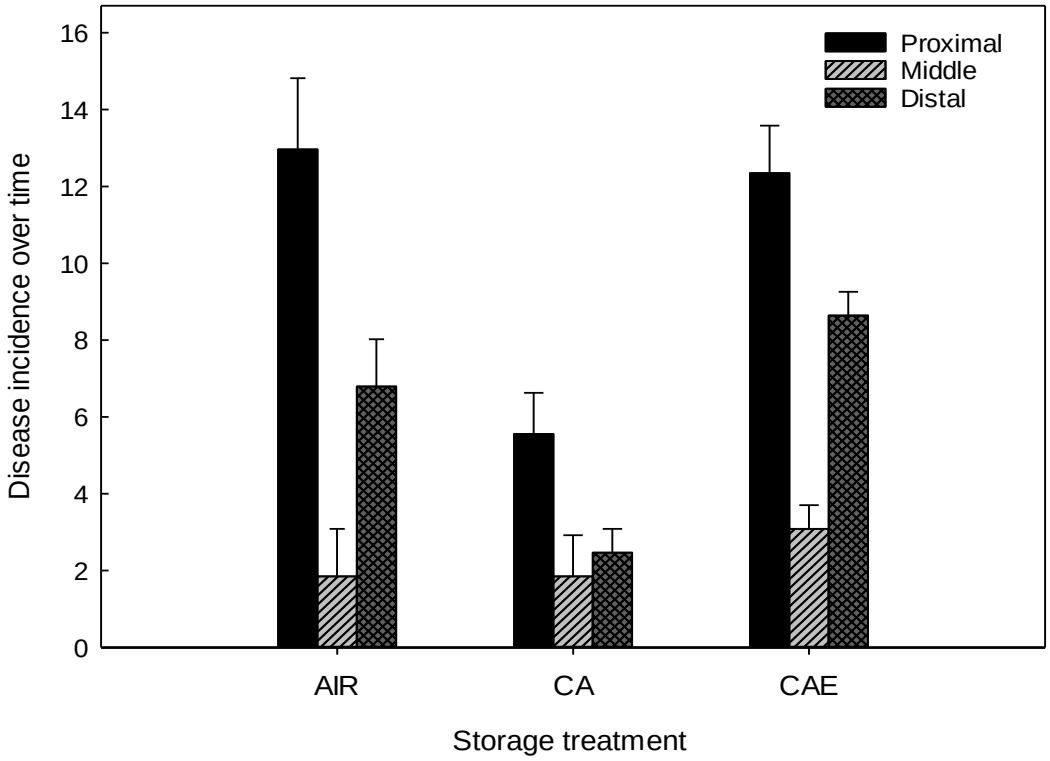


Figure 4-1 The effects of storage treatments: Air (0.003 kPa CO_2 and 21 kPa O_2), CA (5 kPa CO_2 and 8 kPa O_2) and CA supplemented with 0.001 kPa ethylene (CA + Ethylene), on disease incidence across the proximal, middle and distal sections of sweet potatoes at 20˚C for a period of 12 weeks. The error bars represent the standard errors of the weighted means

The storage treatments facilitated mainly the growth of *Fusarium* and *Penicillium* species – increased growth of *Fusarium* with a corresponding decrease in *Penicillium* over time despite spatial difference (Table 4-2). Baseline evaluation showed a 5.4-fold higher level of *Penicillium* species compared to *Fusarium* at the proximal section whereas *Penicillium* quadrupled that of *Fusarium* at the distal section. *Rhizopus stolonifer* accounted for 2.3% of the identified fungal species at the distal section in the baseline, which rose to 11.3% during air storage. *Fusarium* species population on the sweet potatoes more than quadrupled at the proximal section over time across all the storage treatments when cultured in DG-18. However, ethylene supplemented CA storage inhibited the growth of *Fusarium* species on the sweet potato by 0.6-fold compared to air storage. In addition 3.5% of *Aspergillus* species and 17.5% of *Rhizopus* species was recorded during ethylene supplemented CA storage unlike continuous CA treatment which completely inhibited the growth of the fungi.

Table 4-1 Fungal counts (cfu mg^{-1}) of sweet potatoes treated with Air (0.003 kPa CO_2 and 21 kPa O_2), CA (5 kPa CO_2 and 8 kPa O_2) and CA supplemented with 0.001 kPa ethylene (CA + Ethylene), during storage in at 20˚C for 12 weeks. Values represent weighted means and ± standard error and the l.s.d. represents the storage time – treatment interaction effect only

Storage time (weeks)	Treatment	PDA (cfu mg^{-1})	DG-18 (cfu mg^{-1})
0	Baseline	6.62 ± 2.3	6.52 ± 2.2
6	Air	8.78 ± 2.9	8.73 ± 2.7
	CA	1.36 ± 1.0	1.35 ± 1.1
	CA + Ethylene	8.80 ± 2.4	9.17 ± 1.5
12	Air	11.09 ± 2.1	6.40 ± 1.5
	CA	4.76 ± 1.4	3.45 ± 1.7
	CA + Ethylene	33.52 ± 3.6	38.52 ± 6.8
p-value		0.000	0.000
$l.s.d_{(0.05)}$		3.81	2.71

The p-value ($p<0.05$) represents the probability of fungal counts generated by the ANOVA table (see Appendix B) as a result of the interaction between storage time and treatment over time.

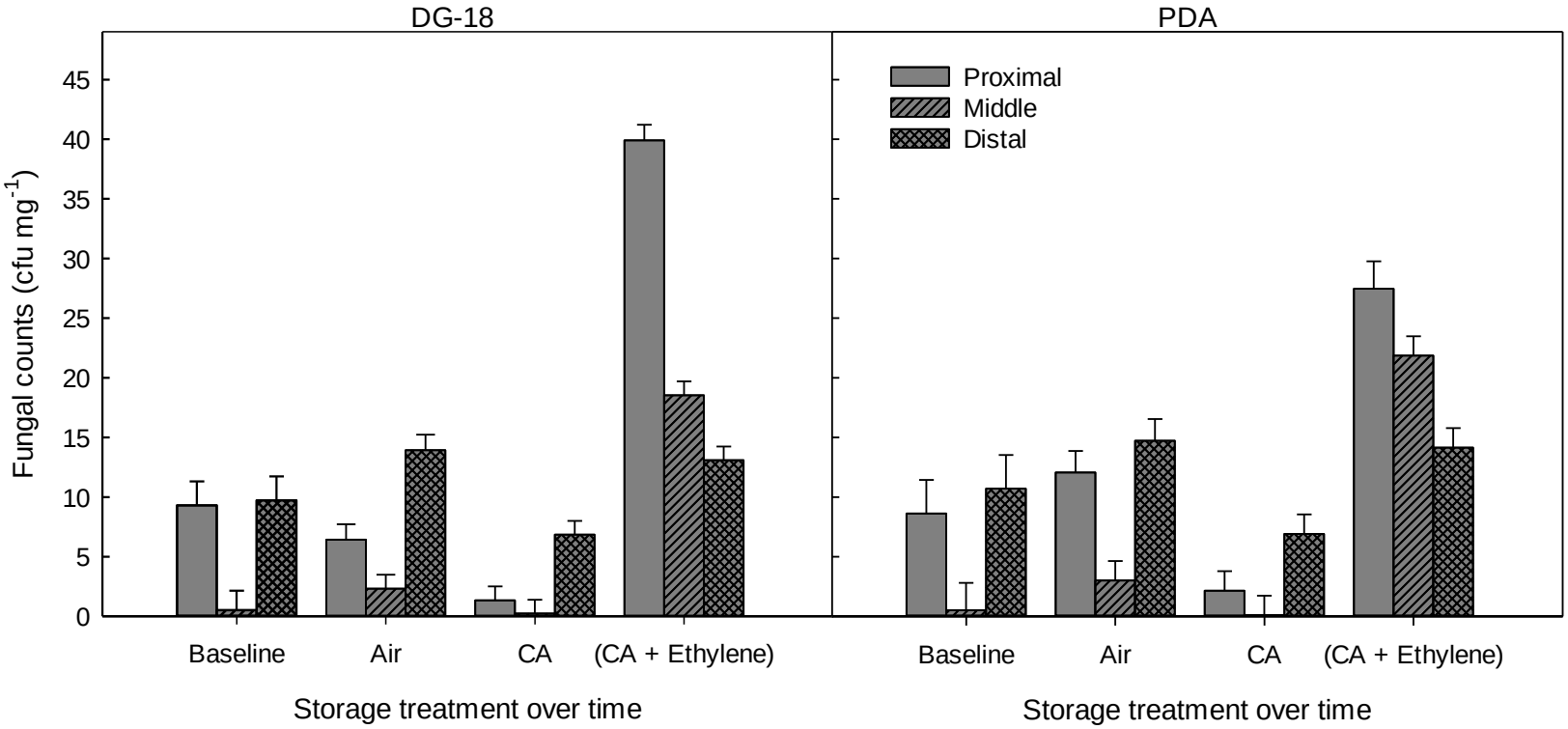


Figure 4-2 The effects of storage treatments: Air (0.003 kPa CO_2 and 21 kPa O_2), CA (5 kPa CO_2 and 8 kPa O_2) and CA supplemented with 0.001 kPa ethylene (CA + Ethylene), on fungal population distribution (cfu mg^{-1}) across the proximal, middle and distal sections of sweet potatoes cultured in Potato Dextrose Agar (PDA) and Dichloran-Glycerol Agar Base (DG-18), at 20˚C for a period of 12 weeks. The error bars represent the standard errors of the weighted means.

Table 4-2 The effects of storage treatments: Air (0.003 kPa CO_2 and 21 kPa O_2), CA (5 kPa CO_2 and 8 kPa O_2) and CA supplemented with 0.001 kPa ethylene (CA + Ethylene), on the proportion of identified fungal species across the proximal, middle and distal sections of sweet potatoes cultured in Dichloran-Glycerol Agar Base (DG-18), at 20˚C for a period of 12 weeks. Values represent the weighted means ± standard errors and the l.s.d. represents treatments – location interaction effects only.

Storage treatment	Location	Percentage fungal species on the stored sweet potato along spatial gradients			
		Fusarium spp	*Penicillium spp*	*Aspergillus spp*	*Rhizopus spp*
Baseline	Proximal	15.34 ± 0.39	83.85 ± 0.49	0.81 ± 0.81	n.d.
	Middle	n.d.	100.00 ± 0.0	n.d.	n.d.
	Distal	17.88 ± 4.27	79.49 ± 6.88	n.d.	2.63 ± 2.63
Air	Proximal	69.65 ± 12.81	30.12 ± 12.81	0.23 ± 0.02	n.d.
	Middle	61.11 ± 5.55	38.89 ± 5.55	n.d.	n.d.
	Distal	67.07 ± 7.35	24.65 ± 2.32	n.d.	11.31 ± 8.21
CA	Proximal	66.67 ± 33.33	33.33 ± 33.33	n.d.	n.d.
	Middle	n.d.	n.d.	n.d.	n.d.
	Distal	86.17 ± 1.98	13.83 ± 1.98	n.d.	n.d.
CA + Ethylene	Proximal	68.22 ± 28.62	31.78 ± 28.62	n.d.	n.d.
	Middle	74.76 ± 8.45	4.17 ± 4.16	3.57 ± 3.57	17.50 ± 11.81
	Distal	40.51 ± 6.60	59.49 ± 6.60	n.d.	n.d.
p-value		0.004	0.115	0.310	0.027

n.d. not detected

The p-value ($p<0.05$) represents the probability of fungal counts generated by the ANOVA table (see Appendix B) as a result of the interaction between storage treatment and spatial orientation of the sweet potato over time.

4.3.2 Potential aflatoxin production and accumulation during storage of sweet potato

Some of the fungi identified (*Aspergillus* species in Table 4-2 above) were potentially mycotoxigenic. Three types of potential aflatoxins: aflatoxin B1 (AFB1), aflatoxin B2 (AFB2) and aflatoxin G1 (AFG1) were detected on the baseline samples and during storage in continuous air and ethylene supplemented CA as shown in Table 4.3 below. There was a differential level ($p < 0.05$) of aflatoxins production and accumulation depending on the storage treatment and the spatial orientation of the sweet potato. Aflatoxin G1 was the most predominant of the three potential strains identified during storage – 105.4 ng g^{-1} and 94 ng g^{-1} – at the distal section of the root under ethylene supplemented CA and continuous air storage respectively. Also CA supplemented with ethylene *c.a.* doubled and increased by 11% AFB1 and AFG1 accumulation respectively at the distal section compared to air storage over time. However, the potential of AFB2 contamination was completely eliminated when treated with ethylene supplemented CA and AFB1 was significantly inhibited at the proximal section over the same time period.

Table 4-3 The effects of storage conditions: Air (0.003 kPa CO_2 and 21 kPa O_2), CA (5 kPa CO_2 and 8 kPa O_2) and CA supplemented with 0.001 kPa ethylene (CA + Ethylene), on the control and management of potential aflatoxins B1, B2 and G1 development on sweet potatoes at 20˚C over a period of 12 weeks. Values represent weighted means ± standard error and the l.s.d. represents treatment effect only.

Storage time (weeks)	Treatment	Location	AFB1(ngg^{-1})	AFB2 (ngg^{-1})	AFG1(ngg^{-1})
0	Baseline	Proximal	8.29 ± 1.49	16.00 ± 2.00	10.92 ± 0.18
6	Air	Distal	2.00 ± 0.2	n.d.	28.25 ± 2.29
	CA + Ethylene	Proximal	n.d.	n.d.	28.65 ± 2.95
12	Air	Distal	6.49 ± 1.33	12.66 ± 0.82	93.89 ± 0.92
	CA + Ethylene	Proximal	0.61 ± 0.31	n.d.	n.d.
		Distal	12.14 ± 0.27	n.d.	105.42 ± 2.37
p-value			0.04011	0.00037	0.000
$l.s.d._{(0.05)}$			2.90	3.05	6.39

The p-value ($p<0.05$) represents the probability of fungal counts generated by the ANOVA table (see Appendix B) as a result of the interaction between storage time, treatment and spatial orientation over time.

4.4 Discussion

The additive treatment of CA to 0.001 kPa ethylene is expected to make the process of inhibiting the adverse factors – of ethylene (e.g. microbial decay) – on sweet potato more effective as was recommended by Amoah et al., (2016). However, this study has shown otherwise due to the possibility that the increased starch hydrolysis (as established in Experiments 1 and 2) during storage may have hindered the capacity of CA in mitigating the adverse effects of ethylene. On the other hand, continuous CA treatment was effective alternative as opposed to ethylene supplemented CA in enhancing the natural disease resistance of the sweet potato (due to the pathogen-environment-host interaction) to fungal attack and consequent decay during storage. Lewthwaite et al. (2013) pointed out that disease susceptibility in sweet potato is further exacerbated by piercing wounds on the root surface. Interestingly, even though tissue disruption i.e. splitting was noticed on sweet potatoes after nine weeks of storage under continuous CA, it was unclear why that did not enhance disease incidence as opposed to CA supplemented with ethylene. As a means of addressing disease incidence, Afek and Orenstein (2003) have reported treatment of sweet potato (cv. 'Georgia Jet') with steam during storage for a period of five months, as an effective method in reducing decay (3% in cured roots and 14% in non-cured compared to 32% and 86% in non-treated cured and non-cured roots respectively). The effects of 1-Methylcyclopropene (1-MCP) has also been reported to have anti-decay effect on sweet potato thereby boosting the natural disease resistance of the root during storage (Amoah and Terry, 2018).

The storage treatments in this study were ineffective in controlling *Fusarium* species development but were effective in controlling *Penicillium* species development over time. The detection of *Rhizopus* soft rot and surface rot as the predominant diseases on the sweet potatoes agrees with earlier reports (Ray and Ravi, 2005; Lewthwaite et al., 2013; Amoah et al., 2016; El Neshawy, 2016) that these two diseases are the most common in tropical conditions. However, infection rates have been shown to be dependent on the sweet potato cultivar, which in this study could not be established because only one cultivar was used. A report by Olaitan (2012) suggested that modified atmosphere storage using a polythene bag of 18µm thickness, can effectively control fungal disease incidence on sweet potato. This study showed that continuous CA was effective in mitigating disease occurrence on the sweet potato, suggesting that deviating

from the regular atmosphere could have positive impact on disease control during storage.

Despite the fact all the fungal species identified in this study were potentially mycotoxigenic, the investigations were limited to the *Aspergillus* isolates (n = 24) for potential aflatoxin contamination. Increased water activity (a_w) (0.99 - 0.96) has been associated with increased production of AFB1 in maize (Abdel-Hadi et al., 2012) and corn (Astoreca et al., 2014) under tropical conditions (25 - 30˚C). It is generally accepted that AFB1 production declines with decreasing water activity. Although the water activity of the sweet potato samples was not determined in this study, the measured relative humidity during storage (85 – 95%) gave an idea of the water activity. The sweet potatoes treated with continuous CA were all free of *Aspergillus* contamination indicating its potential effectiveness in controlling aflatoxin production during storage. Williams et al., (2014) reported effective prevention of *Aspergillus flavus* growth and aflatoxin B1 production in maize as a result of storage in a low oxygen/high CO_2 environment over a period of two months. Likewise, in this study, the diffusion of higher CO_2 levels through the sweet potato root's surface during continuous CA storage may have created an unfavorable environment for the fungi to develop. This therefore implies that the storage of sweet potato under continuous CA can inhibit the potential of these pathogens to induce the production of aflatoxins, which in turn prevent the occurrence of deadly diseases in humans such as liver cancer as well as other health problems in animals. According to Chulze (2010), the hazard analysis and critical control point systems (HACCP) approach can be used as a preventive strategy to reduce the impact of mycotoxin in maize food and feed chains.

4.5 Conclusions

In conclusion, this study has shown that CA storage of sweet potato was effective in controlling *Penicillium* development and consequent root decay. However, *Fusarium* development was not inhibited during storage as the diseased roots were characterised mainly by *Fusarium* surface rot as well as *Rhizopus* soft rot predominantly at the proximal and distal sections. Aflatoxin G1 was the most predominantly produced potential aflatoxin on the sweet potato during storage. The treatment of the roots with CA was very effective in controlling the potential contamination of the studied

aflatoxins during storage unlike the other two storage methods. The biochemical basis surrounding spatial influence on fungal growth and development is not fully explained in this report which warrants further investigation. Despite the fact that in-vitro analyses was not done, the results in this study could be useful in designing effective control strategies for aflatoxins contamination, hence minimising the health risks associated with fungal activity during storage.

4.6 Acknowledgements

My sincere thanks and appreciation go to the Commonwealth scholarship commission for their support in providing me the opportunity to have this great experience. I would also like to thank members of the applied mycology and plant science groups for their technical assistance.

4.7 References

Abdel-Hadi, A., Schmidt-Heydt, M., Parra, R., Geisen, R. and Magan, N. (2012). A systems approach to model the relationship between aflatoxin gene cluster expression, environmental factors, growth and toxin production by *Aspergillus flavus*. *Journal of the Royal Society Interface*, 9, 757–767.

Afek, U., Orenstein, J., and Nuriel, E. (2000). Using HPP (hydrogen peroxide plus) to inhibit potato sprouting during storage. *American Journal of Potato Research*, 77(1), 63–65.

Alamar, M. C., Collings, E., Cools, K. and Terry, L. A. (2017). Impact of controlled atmosphere scheduling on strawberry and imported avocado fruit, *Postharvest Biology and Technology*, 134, 76–86

Amoah, R. S., Landahl, S., and Terry, L. A. (2016). The timing of exogenous ethylene supplementation differentially affects stored sweet potato roots. *Postharvest Biology and Technology*, 120, 92–102

Amoah, R.S. and Terry, L.A. (2018). 1-Methylcyclopropene (1-MCP) effects on natural disease resistance in stored sweet potato. *Journal of the Science of Food and Agriculture*, in Press

AOAC International (2000). AOAC official methods of analysis. AOAC official method 997.13. Glycoalkaloids (a-solanine and a-chaconine) in potato tubers. In: Trucksess MW (ed) Natural toxins. AOAC, Gaithersburg, p. 63–65.

Astoreca, A., Vaamonde, G., Dalcero, A., Marin, S. and Ramos, A. (2014). Abiotic factors and their interactions influence on the co-production of aflatoxin B(1) and cyclopiazonic acid by *Aspergillus flavus* isolated from corn. *Food Microbiology*, *38*, 276–83.

Cantwell, M. and Suslow, T. (2013). Sweet potato: Recommendations for maintaining postharvest quality. Department of Plant Science, University of California. [Available at http://postharvest.ucdavis.edu/pfvegetable/Sweetpotato ; accessed: 28th October, 2014]

Chulze, S.N. (2010). Strategies to reduce mycotoxin levels in maize during storage: a review. *Food Additives and Contaminants*, 27(5), 651-657.

Delate, K. M. and Brecht, J. K. (1989). Quality of tropical sweet potatoes exposed to controlled atmosphere treatments for postharvest insect control. *Journal of the American Society for Horticultural Science*, 114, 963–968.

Delate, K. M., Brecht, J. K. and Coffelt, J. A. (1990). Controlled atmosphere treatments for control of sweet potato weevil (Coleoptera: *Curculionidae*) in stored tropical sweet potatoes. *Journal of Economic Entomology*, 82, 461–465.

El Neshawy, S. M. (2016). Management of the whole process of fresh Egyptian sweet potato prepared for export against soft rot. *Acta Horticulturae*, 1144, 417-422

Holmes,G.J. and Stange,R.R.(2002). Influence of Wound Type and Storage Duration on Susceptibility of Sweet Potatoes to Rhizopus Soft Rot. *Journal of Plant Disease,* 86, 345-348

Kays, S.J. and S.E. Kays. (1998). Sweet potato chemistry in relation to health. p. 231-272. In: Sweet potato Production Systems toward the 21st Century. Kyushu National Agri. Expt. Station., Miyakonojo, Miyazaki, Japan.

Lewthwaite, S. L., Wright, P. J. and Triggs, C. M. (2013). Sweet Potato Cultivar Susceptibility to Postharvest Soft Rot Caused by *Rhizopus stolonifer*. *New Zealand Plant Protection*, 66, 223–228.

Olaitan, O. O. (2012). Bio-deterioration of sweet potato (*Ipomoea batatas* L.) in storage, inoculation-induced quality changes and control by modified atmosphere. *Journal of Applied Science and Environmental Management*, 16(2), 189–193.

Pedreschi, R., Lurie, S., Hertog, M., Nicolaï, B., Mes, J. and Woltering, E. (2013). Post-harvest proteomics and food security. *Proteomics*, 13, 1772–1783.

Ray, R. C. and Ravi, V. (2005). Postharvest spoilage of sweetpotato in tropics and control measures. *Critical Reviews in Food Science and Nutrition*, 45(7–8), 623–644.

Rees, D., van Oirschot, Q. E. ., Amour, R., Rwiza, E., Kapinga, R., and Carey, T. (2003). Cultivar variation in keeping quality of sweet potatoes. *Postharvest Biology and Technology*, 28(2), 313–325.

Samson, R. A. Houbraken, J. Thrane, U., Frisvad, J. C. and Andersen, B. (2010) *Food and Indoor Fungi*. CBS Laboratory Manual Series, Volume: 2; CBS-KNAW-Fungal Biodiversity Centre, Utretcht, The Netherlands

Williams, S. B., Baributsa, D. and Woloshuk, C. (2014). Assessing Purdue Improved Crop Storage (PICS) bags to mitigate fungal growth and aflatoxin contamination. *Journal of Stored Products Research*, 59, 190–196.

5 MOLECULAR REGULATION OF METABOLIC BIOSYNTHESIS IN SWEET POTATO STORED UNDER CONTROLLED ATMOSPHERE AND ETHYLENE CONDITIONS

S. Sowe, R. Tosetti, S. Landahl, and L.A. Terry*

Plant Science Laboratory, Cranfield University, Bedfordshire, MK43 0AL, United Kingdom
*Corresponding author - E-mail: l.a.terry@cranfield.ac.uk ; Tel: +44 (0)1234 750111

Abstract

This study reports on how storage under supplemental ethylene and CA influence the molecular regulation of metabolic activities in sweet potato root. Sweet potato samples (cultivar: 06-52; known as Belle Vue in the USA) were stored under CA (5kPa CO_2 and 8kPa O_2); air (0.003kPa CO_2 and 21kPa O_2); ethylene (0.001kPa) and the transitions from continuous ethylene to air storage and vice versa at 20˚C for nine weeks. Biochemical assays (non-structural carbohydrates, phenolics and plant growth hormones) were carried out using the high-performance liquid chromatograph (HPLC) and LCMS for the phytohormones, whilst the molecular assays were done using the quantitative polymerase chain reaction (qPCR) technique.

In as much as the ethylene supplementation was successful in supressing sprouting, the contents of phenolics and sugars increased which undermines its positive aspects. Air and CA treatments exhibited a similar pattern during storage. The accelerated synthesis of these metabolites was as a result of the stressful environment created during storage which translated into increased respiratory energy and water loss in the sweet potato. The complete inhibition of sprouting during storage could also be attributed to decreased biosynthesis of the cytokinin trans-ZR in the sweet potato due to ethylene supplementation. Also ethylene induced rise in phenolics corresponded with an increased relative expression of ERF in the proximal section unlike the distal section of the sweet potato during storage irrespective of the root tissue. The ERF gene was more expressed towards the end of storage whilst EIN2 was more expressed at the beginning which suggested that EIN2 was actively repressed during storage particularly under CA treatment. The increased relative expression of the ERF gene in the flesh at the proximal

section during ethylene storage, did not translate in a concurrent increase in the reducing sugars.

Keywords: *Ipomoea batatas*; non-structural carbohydrates; phytohormones; genes; primers

5.1 Introduction

The roots of sweet potato (*Ipomoea batatas*) are perishable and therefore require effective handling and storage methods in order to maximise postharvest life. Flushing stored sweet potatoes with exogenous ethylene (10 µL L^{-1}) and timing has been used to address the problem of postharvest deterioration, yielding some positive results, e.g. sprout control (Cheema et al., 2013; Amoah et al., 2016). However, some adverse effects were observed as a result of the use of exogenous ethylene viz: increased disease incidence at the proximal section, a rise in weight loss increased respiration rates and increased sugars. The regulation of ethylene in plant tissues triggers signals which elicit several physiological responses in activating target genes. According to Iqbal et al. (2013), plants' tissue response to ethylene is triggered by the activated complex resulting from the binding of ethylene to a receptor. This response then leads to a chain of reactions within the plant tissues which in turn results in a wide range of physiological changes. Ethylene response factors (ERFs) constitute one of the largest transcription factors and are important regulators of low oxygen tolerance in plants as well as the biosynthesis of ethylene (Zhang et al., 2009; Cukrov et al., 2015). Ethylene insentitive 2 (EIN2) has also been shown to be a key player in the ethylene signalling pathway as it expression activates the response of the ethylene in plant tissues. The EIN2 protein accumulates as a result of ethylene treatment which is required for stabilization of the biosynthetic pathway process (Merchante et al., 2013).

Limiting the biosynthesis of ethylene has been carried out using many strategies including the application of aminoethoxyvinylglycine (AVG) (Cheema 2010), 1-methylcyclopropane (1-MCP) (Amoah and Terry, 2018) and CA storage. Controlled atmosphere storage has not been used in sweet potato as much as it should. Its application has been mainly for weevil control Delate et al. (1990) and therefore needs to be explored further. In as much as the adverse effects of ethylene could be inhibited by CA storage, the concept is yet to be fully understood regarding sweet potato. Therefore understanding the molecular processes regulating the biochemical dynamics

during storage of the roots is of particular importance. Hence this study critically investigated the effects of ethylene supplementation and CA on specific metabolites (sugars, phenolic compounds and plant growth regulators) and the molecular triggers underpinning the process during storage of sweet potato. This information may provide a greater understanding on the gene expression patterns in the different root tissues during storage as a result of the studied treatments.

5.2 Materials and methods

5.2.1 Plant materials and experimental design

The sweet potato roots (*Ipomoea batatas* L., cultivar: 06-52; known as Belle Vue in the USA) were harvested on 25th November, 2016 and delivered at Cranfield University on 21st February, 2017 as explained in chapter three, section 3.2.1. The sweet potatoes were subsequently stored for nine weeks at 20˚C as described under the following conditions: (1) continuous CA (5 kPa CO_2 and 8 kPa O_2); (2) continuous ethylene (0.001 kPa); (3) CA supplemented with 0.001 kPa ethylene and (4) continuous air (0.003 kPa CO_2 and 21 kPa O_2). At six weeks of storage, a transitional phase across storage treatments was introduced: (5) continuous ethylene followed by air and vice versa as described by Amoah et al. (2016). A schematic representation of the experimental set-up is shown in Figure 5-1 below.

5.2.2 Biochemical assessments during storage

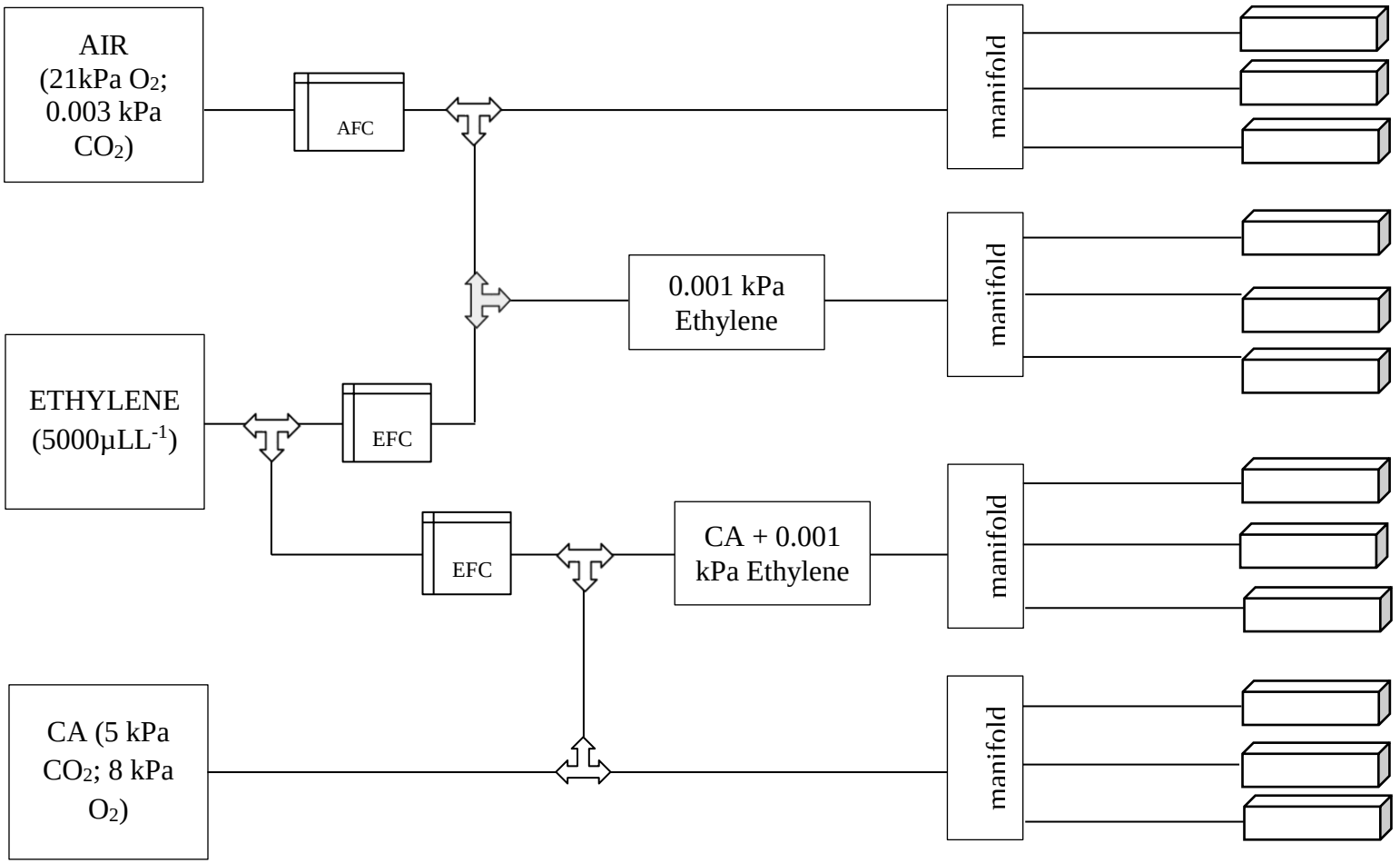
Samples preparations for biochemical assays were carried out following the method described by Amoah et al., (2016) with modifications as explained in chapter three, section 3.2.4.

5.2.2.1 Extraction and quantification of NSCs and phenolic compounds

Extraction of the NSCs and phenolic compounds was done using the method described by Amoah et al., (2016) as explained in chapter three, section 3.2.4.1 and 3.2.4.2. Quantification of individual NSCs was carried out with the HPLC – Refractive Index Detector (RID) using Agilent ChemStation software.

5.2.2.2 Extraction and quantification of phyto-hormones

Phyto-hormones were extracted and quantified as described in chapter three, section 3.2.4.3.



EFC: Ethylene Mass Flow Controller; AFC: Air Mass Flow Controller

Figure 5-1 Schematic representation of treatments gas flow into storage boxes of sweet potatoes at 20˚C over a period of nine weeks

5.2.3 Primer design and selection

The primers used in this study (Table 5.1 below) were designed using the protein sequences of the selected genes, generated from the software developed by the National Centre for Biotechnology Information (NCBI). The designs were done by running the FASTA files of the selected gene nucleotide using Primer3Plus software. The designed primers were then checked and selected based on the product size (100 – 350 bp), GC content (50 – 60%), and annealing temperature (55 – 65°C), using the software developed by Premier Biosoft International (Beacon Design – Free edition).

Table 5-1 List of primers used for the quantitative polymerase chain reaction (qPCR) analyses to study the expression of Ethylene insensitive2 (EIN2) and Ethylene response factors (ERF) genes in sweet potato during storage. Actin was used as the housekeeping gene against which the sample genes above were compared

Primers	Forward sequence	Reverse sequence
Actin	5'TGTTAGCAACTGGGATGATATGG3'	5'GGATAGCACAGCCTGAATAGC3'
EIN2	5'CGAAGGTTCTGACTGGCTGT3'	5'TCTGGCCTGCTTTCCATGAG3'
ERF	5'GTCATAGCGACTGCGATTCT3'	5'GGCAGGTTGAGATCGAAAGA3'

5.2.4 Total ribonucleic acid (RNA) extraction

Total RNA was extracted using eight grams of the freeze dried sweet potato samples as described by Untergasser's RNA Miniprep Cetyl Trimethyl Ammonium Bromide (CTAB) protocol, version 1.0 (Untergrasser's Lab, Germany) together with a modified Qiagen RNeasy Plant mini kit protocol (QIAGEN Ltd., UK). The flesh and peel samples of the sweet potato from the proximal and distal sections, in three technical replicates, were assessed for total RNA (n = 168). To avoid DNA contamination, the RNA samples were treated with DNase. The quantity and purity of the total RNA were evaluated using the Experion RNA StdSens Analysis Kit (Bio-Rad Laboratories Ltd., UK).

5.2.5 Analysis of ERF and EIN2 genes expression by quantitative polymerase chain reaction (qPCR)

The extracted RNA samples were each reverse transcribed to produce cDNAs using the Quick – Start protocol of the QuantiTect® Reverse Transcription Kit (QIAGEN Ltd., UK). The quality of the cDNAs was validated by normal PCR using primers

corresponding to the studied genes in Table 5.1 above, as described by Lin et al., (2009) with slight modifications. Quantitative PCR analysis was then performed in a 2013 Real-Time (RT) PCR System (Bio-Rad Laboratories Ltd., UK) using the Quick – Start protocol of QuantiTect® SYBR® Green PCR Kit (QIAGEN Ltd., UK). The amplification reaction was performed using a total volume of 20 µL in each well of a 96 wells plate (45 wells for the sample gene with a corresponding 45 wells for the reference gene and three wells of NTC each for sample and housekeeping genes) under the following conditions: denaturation at 95°C for 15 min, followed by 40 cycles of the routine (94°C for 15 s, 60°C for 30s and 72°C for 30s), then by a final extension at 72°C for 5 min. The relative normalized expression (ΔΔCq) of each of the studied genes was obtained using the inbuilt Bio-Rad CFX manager™ software, version 3.1 (admin), of the RT – PCR system. The relative expression levels of each of the sample genes (i.e. ERF and EIN2) were then calculated against the housekeeping gene (Actin), as described by Ponniah et al., (2017) with modifications using the ΔΔCq method.

5.3 Results

5.3.1 Changes in non-structural carbohydrates (NSCs) during storage

The concentrations of NSCs significantly varied in the flesh and peel tissues of the sweet potato irrespective of treatment applied. The flesh concentrations of the reducing sugars tripled that of the peel (Figure 5-2). Ethylene supplementation inhibited flesh monosaccharides accumulation by half ($p<0.05$) compared to air storage. However, the reducing sugars increased their accumulation by *c.a.* equivalent amounts (1.7-fold) in the flesh as a result of truncating ethylene supplementation compared to its uninterrupted application. Air storage followed by ethylene showed a similar pattern in reducing sugars accumulation. On the other hand flesh sucrose content tripled and quadrupled during air and ethylene storage respectively whereas continuous CA with and without supplemented ethylene boosted sucrose accumulation by five-fold during storage. The reducing sugars doubled the baseline concentration in the peel after six hours of storage. However, the peel fructose concentration during storage under ethylene, continuous CA and continuous ethylene supplemented CA significantly declined by four, seven and five-folds, with a concomitant peel glucose decrease of 1.4, 1.4 and 1.2-folds, respectively over time. In contrast, continued exposure of the sweet

potato to ethylene supplemented CA tripled ($p<0.05$) peel sucrose accumulation whilst the disaccharide stabilized to baseline level in the peel as a result of truncating ethylene supplementation.

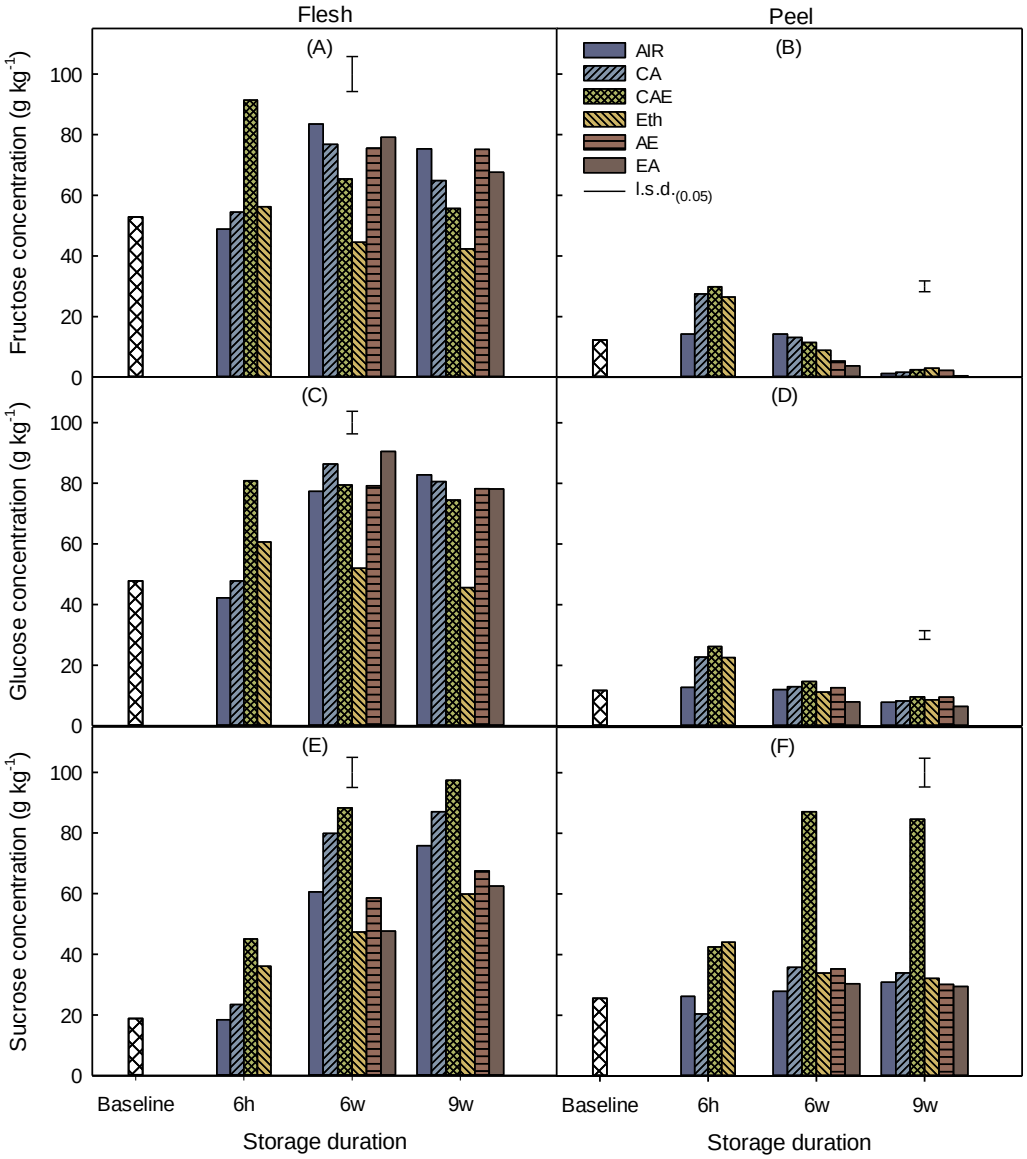


Figure 5-2 The effects of storage in – air (21 kPa O_2 and 0.003 kPa CO_2); controlled atmosphere (CA: 8 kPa O_2 and 5 kPa CO_2); controlled atmosphere supplemented with 0.001 kPa ethylene (CAE); continuous ethylene (Eth: 0.001 kPa); air treatment followed by continuous ethylene (AE); continuous ethylene followed by air (EA) on the sugar contents of sweet potatoes (dry weight basis), at 20˚C over 12 weeks. The l.s.d. $_{(0.05)}$ bars shown in the figures above represent that for storage time-treatment interactions during storage. Sampling was done on the baseline, at six hours (6h), at six weeks (6w) and at nine weeks (9w) of storage. The transition across the treatments occurred at six weeks of storage.

5.3.2 The metabolism of phenolic compounds during sweet potato storage

The distribution of the studied phenolic compounds indicated a disproportionately higher concentration in the peel than in the flesh of the sweet potato (Figure 5-3 and 5-4). This study also showed that Isochlorogenic acid B and chlorogenic acid were the most abundant phenolics in the peel and flesh respectively. In as much as peel chlorogenic acid accumulation was inhibited during storage, ethylene supplemented CA significantly accelerated its accumulation in the flesh as did flesh Isochlorogenic acid B (1.5-fold). Air storage as well as supplemental ethylene stabilized flesh chlorogenic acid accumulation but Isochlorogenic acid B was doubled during air storage. The content of flesh Isochlorogenic acid A during continuous ethylene supplementation doubled and tripled that of air and CA storage respectively.

On the other hand, air storage resulted in an exponential increase in peel Isochlorogenic acid C (*c.a.* seven-fold) as did CA supplemented with ethylene (*c.a.* nine-fold) over time. Continuous ethylene supplementation doubled the content of Isochlorogenic acid C accumulated during CA storage. Similarly, truncating ethylene treatment boosted flesh Isochlorogenic acid B by 1.5-fold and that of Isochlorogenic acid C to a lesser extent compared to continuous ethylene. Conversely, air storage followed by ethylene treatment inhibited flesh Isochlorogenic acid A by half compared to continuous ethylene storage.

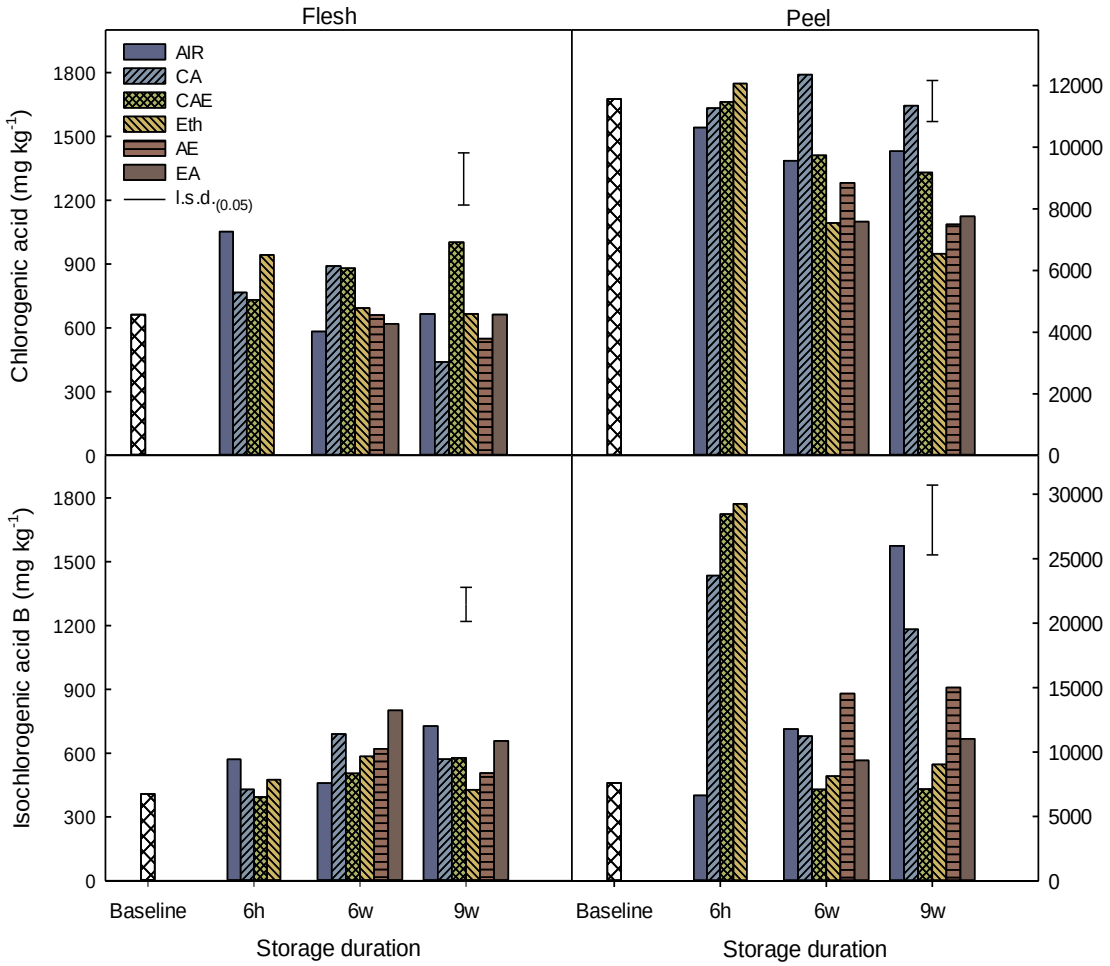


Figure 5-3 The effects of storage – air (21 kPa O_2 and 0.003 kPa CO_2); controlled atmosphere (CA: 8 kPa O_2 and 5 kPa CO_2); controlled atmosphere supplemented with 0.001 kPa ethylene (CAE); continuous ethylene (Eth: 0.001 kPa); air treatment followed by continuous ethylene (AE); continuous ethylene followed by air (EA) on the flesh and peel contents (dry weight basis) of chlorogenic acid and Isochlorogenic acid B of sweet potatoes, at 20˚C over 12 weeks. The l.s.d.$_{(0.05)}$ bars shown in the figures above represent that for storage time-treatment interactions during storage. Sampling was done on the baseline, at six hours (6h), at six weeks (6w) and at nine weeks (9w) of storage. The transition across the treatments occurred at six weeks of storage.

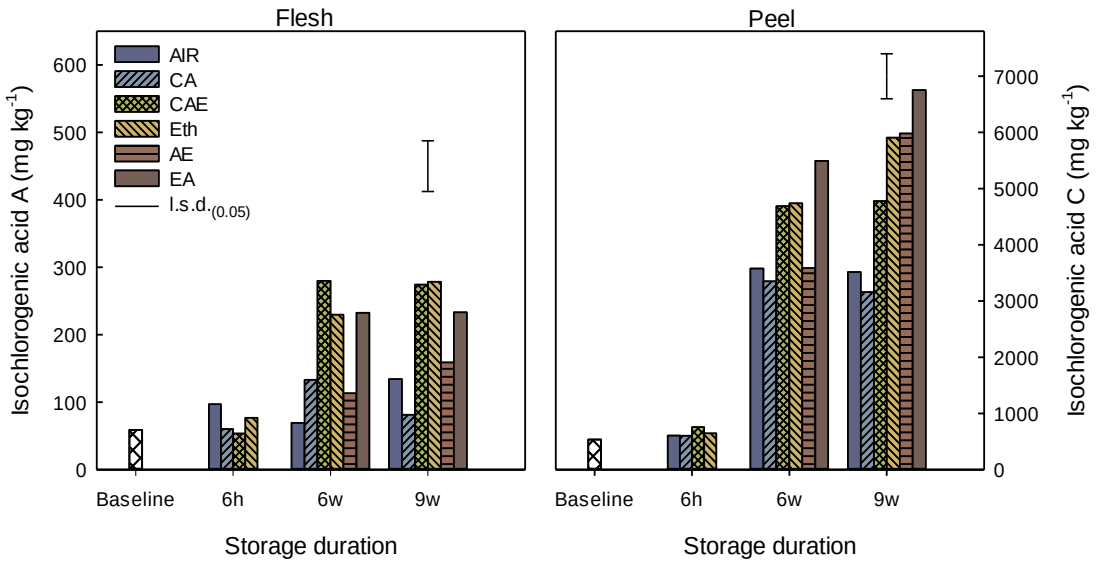


Figure 5-4 The effects of storage in – air (21 kPa O_2 and 0.003 kPa CO_2); controlled atmosphere (CA: 8 kPa O_2 and 5 kPa CO_2); controlled atmosphere supplemented with 0.001 kPa ethylene (CAE); continuous ethylene (Eth: 0.001 kPa); air treatment followed by continuous ethylene (AE); continuous ethylene followed by air (EA) on the flesh content (dry weight basis) of Isochlorogenic acid 'A' and the peel content of Isochlorogenic acid 'C', of sweet potatoes, at 20˚C over 12 weeks. The $l.s.d._{(0.05)}$ bars shown in the figures above represent that for storage time-treatment interactions during storage. Sampling was done on the baseline, at six hours (6h), at six weeks (6w) and at nine weeks (9w) of storage. The transition across the treatments occurred at six weeks of storage.

5.3.3 Phyto-hormonal dynamics in sweet potato during storage

Like trans-zeatin riboside (trans-ZR) in the peel, flesh abscisic acid (ABA) concentration of the sweet potato decreased during storage except in the case of the transitions from air to ethylene and vice versa. The baseline concentration of flesh ABA halved during storage under air and ethylene respectively whereas CA storage dropped the concentration by one-third over time (Table 5-2). Ethylene supplementation *c.a.* tripled phaseic acid (PA) accumulation compared to air storage despite the spatial difference. In contrast, CA storage largely inhibited the biosynthesis of PA irrespective of the root tissue as opposed to ethylene supplementation over time (Table 5-3). Truncating ethylene supplementation inhibited the accumulation of PA whilst enhancing ABA concentration compared to continuous ethylene irrespective of the root tissue. The biosynthesis of ABA and trans-ZR in the peel exhibited a reciprocating phenomenon during storage. There was an increase in peel ABA with a concomitant decrease in trans-ZR (Figure 5-5) over time. The proximal peel concentration of trans-

ZR tripled during ethylene supplementation compared to continuous air storage over time. Also this study showed that truncating ethylene had a differential effect trans-ZR dynamics along the spatial gradients of the sweet potato.

Table 5-2 Abscisic acid concentration (ng g^{-1} dry weight basis) of sweet potato roots treated with – air (21 kPa O_2 and 0.003 kPa CO_2), controlled atmosphere (CA: 8 kPa O_2 and 5 kPa CO_2), ethylene (0.001 kPa), air followed by ethylene and ethylene followed by air – during storage at a temperature of 20˚C over a period of 12 weeks. Values represent the square root of the least weighted mean ± standard error and the l.s.d. is for treatment effects only.

Storage treatment	Flesh	Peel
Baseline	48.5 ± 2.2	23.9 ± 5.8
Air	22.4 ± 1.6	37.7 ± 4.2
Controlled Atmosphere	15.5 ± 1.6	32.6 ± 4.1
Ethylene	22.3 ± 1.5	34.6 ± 4.0
Air followed by Ethylene	74.4 ± 2.5	36.0 ± 6.8
Ethylene followed by Air	129.0 ± 2.8	58.5 ± 6.6
p value	0.000	0.001
$l.s.d._{(0.05)}$	3.72	11.02

The p-value ($p<0.05$) represents the probability of ABA biosynthesis on the flesh and peel tissues of the sweet potato generated by the ANOVA table (see Appendix C) as a result of the interaction between storage time and treatment over time.

Table 5-3 Phaseic acid concentration (ng g^{-1} dry weight basis) of sweet potato roots treated with – air (21 kPa O_2 and 0.003 kPa CO_2), controlled atmosphere (CA: 8 kPa O_2 and 5 kPa CO_2), ethylene (0.001 kPa), air followed by ethylene and ethylene followed by air – during storage at a temperature of 20˚C over a period of 12 weeks. Sampling was done on the baseline, at six hours (6h), at six weeks (6w) and at nine weeks (9w) of storage. The transition across the treatments occurred at six weeks of storage. Values represent the square root of the least square mean and the l.s.d. is for storage time-treatment-location interaction effects only.

Storage time	Storage treatment	Flesh		Peel	
		Proximal	Distal	Proximal	Distal
0	Baseline	22.8 ± 22.7	102.5 ± 25.1	242.8± 145.3	449.8 ± 71.9
6h	Air	104.2 ± 21.6	83.5 ± 10.5	227.2 ± 113.6	407.0 ± 44.3
	CA	115.5 ± 1.2	209.4 ± 29.8	476.8 ± 60.7	570.3 ± 42.9
	Ethylene	187.1 ± 22.4	120.5 ± 12.1	640.6 ± 96.8	309.6 ± 66.3
6w	Air	43.6 ± 4.0	45.2 ± 6.7	196.2 ± 25.9	189.9 ± 15.9
	CA	49.7 ± 10.7	90.4 ± 25.7	105.0 ± 29.8	247.6 ± 82.8
	Ethylene	100.7 ± 1.8	78.3 ± 4.7	286.7 ± 79.5	278.0 ± 24.7
	Air followed by Ethylene	141.3 ± 21.7	176.6 ± 1.6	348.8 ± 38.3	349.5 ± 83.3
	Ethylene followed by Air	133.1 ± 40.7	129.4 ± 15.9	271.9 ± 114.6	292.1 ± 30.5
9w	Air	50.6 ± 22.2	32.3 ± 0.9	170.5 ± 26.0	149.7 ± 2.7
	CA	42.3 ± 5.4	43.2 ± 2.9	195.3 ± 36.7	182.7 ± 26.0
	Ethylene	161.8 ± 10.9	228.4 ± 46.2	446.5 ± 35.5	527.0 ± 92.4
	Air followed by Ethylene	85.2 ± 12.9	66.0 ± 19.9	330.0 ± 72.9	344.3 ± 55.7
	Ethylene followed by Air	117.9 ± 37.3	97.3 ± 5.4	357.8 ± 120.1	350.6 ± 20.9
p-value		0.012		0.031	
l.s.d.		51.87		194.71	

The p-value ($p<0.05$) represents the probability of the biosynthesis of Phaseic acid on the flesh and peel tissues along the spatial gradients of the sweet potato generated by the ANOVA table (see Appendix C) as a result of storage treatment over time.

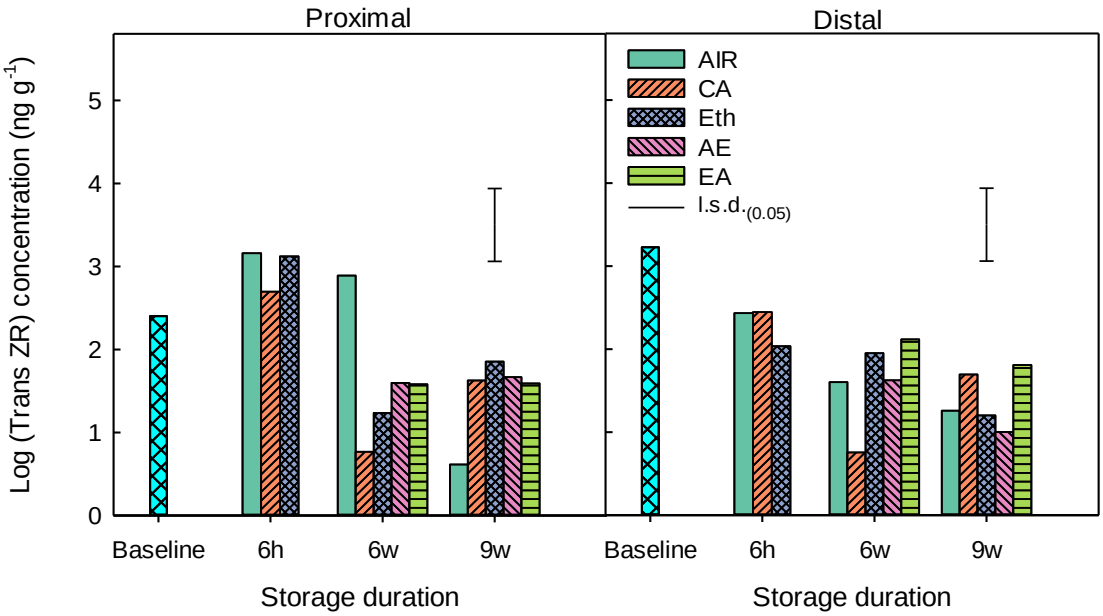


Figure 5-5 The effects of storage in – air (21 kPa O_2 and 0.003 kPa CO_2); controlled atmosphere (CA: 8 kPa O_2 and 5 kPa CO_2); controlled atmosphere supplemented with 0.001 kPa ethylene (CAE); continuous ethylene (Eth: 0.001 kPa); air treatment followed by continuous ethylene (AE); continuous ethylene followed by air (EA) on the peel concentrations (dry weight basis) of trans zeatin riboside (trans-ZR) of sweet potatoes, at 20˚C over 12 weeks. Sampling was done on the baseline, at six hours (6h), at six weeks (6w) and at nine weeks (9w) of storage. The transition across the treatments occurred at six weeks of storage. Values represent Log of least square means and the l.s.d.$_{(0.05)}$ bars shown in the figures above represent that for storage time-treatment-location interactions during storage.

5.3.4 The relative expression of ERF and EIN2 genes during storage

Gene expression results in this study indicated that ERF was up-regulated towards the end of storage whilst EIN2 was up-regulated at the beginning of storage. The relative expression of ERF in the proximal flesh of sweet potato was up-regulated by *c.a.* five-fold during CA storage and tripled during air storage. On the contrary the EIN2 gene was down-regulated significantly over time irrespective of the spatial orientation and storage treatment. On the distal section of the sweet potato, ERF was down-regulated in the flesh irrespective of the treatment applied except for air storage where the relative expression of the gene *c.a.* tripled over time (Figure 5-6). Also ethylene supplementation halved and tripled the relative expression of ERF and EIN2 at the distal flesh respectively after six hours of storage but both genes were subsequently down-regulated over time. However, the flesh expressions of the genes were down-regulated at the proximal section over the same time period as opposed to the peel during ethylene storage. Additionally, ethylene supplementation followed by air storage, upregulated proximal flesh ERF by half and that in the peel by five-fold compared to continuous ethylene supplementation. In contrast the transition from air to ethylene largely down-regulated the studied genes during storage. Similarly, CA storage tripled the relative expression of EIN2 at the proximal peel of the sweet potato after six hours but was steeply down regulated afterwards (Figure 5-7).

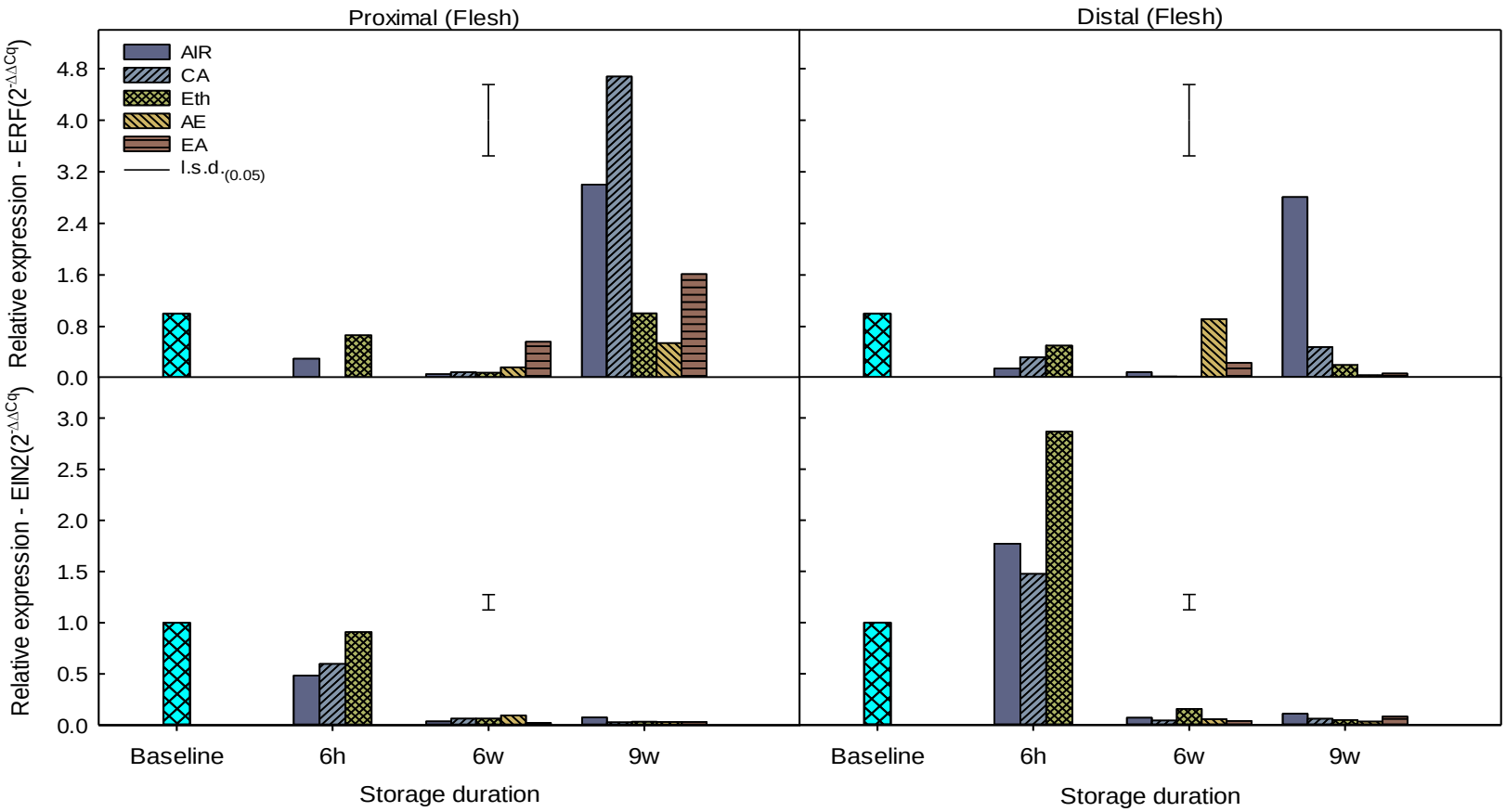


Figure 5-6 The effects of storage conditions: air (21 kPa O_2 and 0.003 kPa CO_2); controlled atmosphere (CA: 8 kPa O_2 and 5 CO_2) and ethylene (0.001 kPa), on the relative expression of ERF and EIN2 genes in the flesh (dry weight basis) of sweet potato at 20˚C over a period of 12 weeks. Sampling was done on the baseline, at six hours (6h), at six weeks (6w) and at nine weeks (9w) of storage. The transition across the treatments occurred at six weeks of storage. The l.s.d. bars represent that of the overall interaction of storage time-treatment-location during storage.

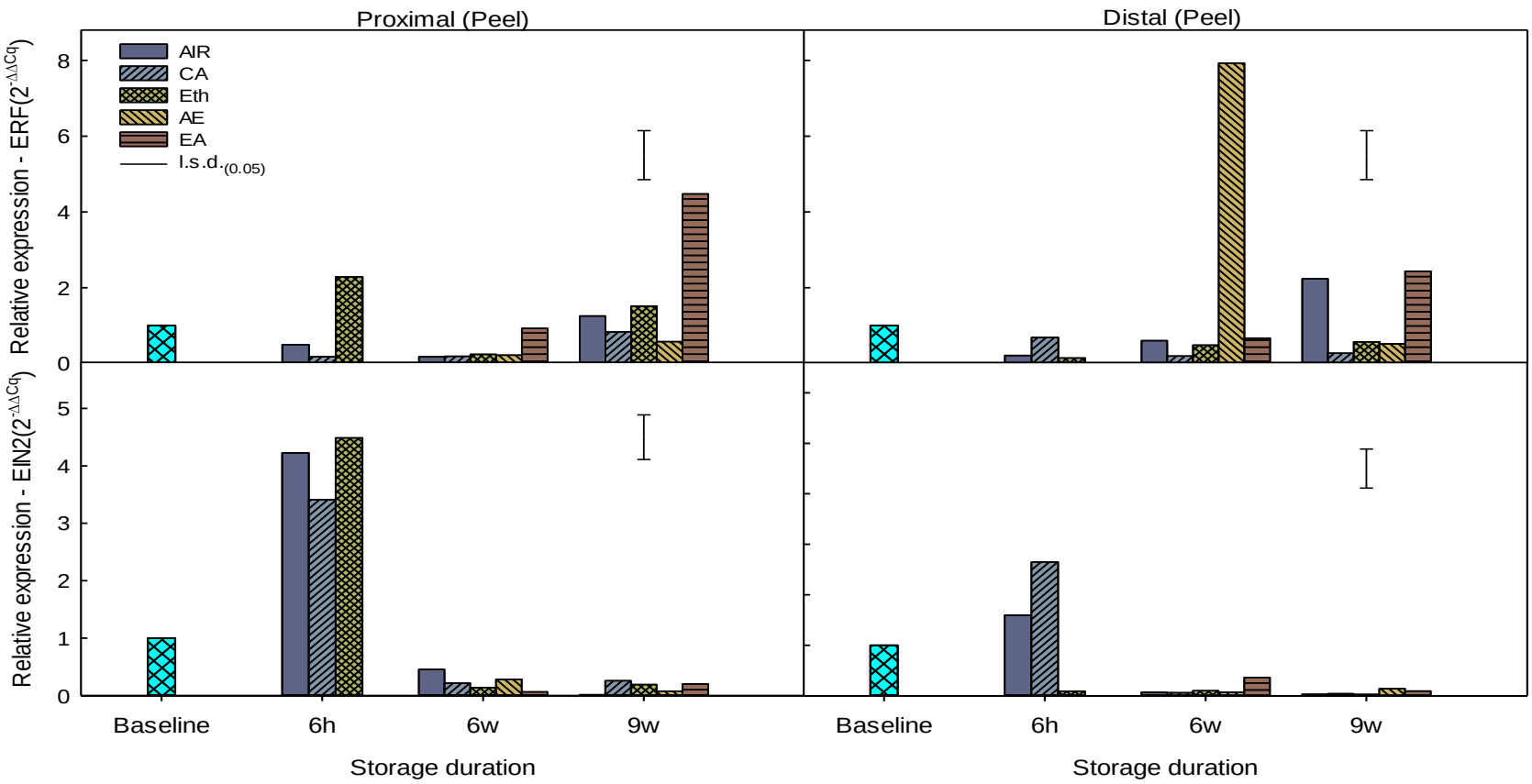


Figure 5-7 The effects of storage conditio conditions: air (21 kPa O_2 and 0.003 kPa CO_2); controlled atmosphere (CA: 8 kPa O_2 and 5 CO_2) and ethylene (0.001 kPa), on the relative expression of ERF and EIN2 genes in the peel (dry weight basis) of sweet potato at 20˚C over a period of 12 weeks. Sampling was done on the baseline, at six hours (6h), at six weeks (6w) and at nine weeks (9w) of storage. The transition across the treatments occurred at six weeks of storage. The transition across the treatments occurred at six weeks of storage. The l.s.d. bars represent that of the overall interaction of storage time-treatment-location during storage.

5.4 Discussion

5.4.1 The effects of gene regulations on the metabolisms of non-structural carbohydrates (NSCs) and polyphenols in stored sweet potato

Results from this study indicated that continuous ethylene supplementation (0.001 kPa) during storage decreased monosaccharides (fructose and glucose) concentration with a concurrent increase in sucrose concentration irrespective of the root tissue. This agrees with earlier findings by Cheema et al., (2013) and Amoah et al., (2016) of reduced monosaccharides accumulation in sweet potato, as a result of supplemental ethylene. The opposite effect on monosaccharides has been reported as a result of ethylene treatment in stored potato (Foukaraki et al., 2016b). Fructose accumulation in the flesh of the non-ethylene treated root as is the case in this study concurs with Adu-Kwarteng et al. (2014) who observed a similar pattern in stored sweet potatoes under ambient conditions. The rise in sucrose could be due to starch hydrolysis triggered by the activation of sucrose phosphate synthase in the root tissues of the sweet potato (Li and Zhang, 2003; Tao et al., 2012; Ponniah et al., 2017). Unlike the monosaccharides in the peel, this study revealed a significantly enhanced accumulation of the NSCs over time as a result of air and CA treatments. Interestingly CA and air treatments also up-regulated the relative expression of the ERF gene at the proximal section of the sweet potato flesh tissue during storage. The mechanisms surrounding this relationship between NSCs and the ERF gene is not clear. The tight regulation of genes has also been associated with reduced starch degradation in sweet potato (Shekhar et al., 2015). However, the fact that ethylene supplementation inhibited the metabolism of the NSCs compared to the other treatments may suggest a link to tighter regulation of the ERF gene.

On the other hand, this study also showed that the accumulation of phenolics was boosted over time except for peel chlorogenic acid. Phenolics have been associated with their facilitation of wound healing and also their likelihood to aiding natural resistance against pathogens (Duvivier et al., 2010; Jung et al., 2011). Meanwhile, selective influence in the accumulation of the individual phenolics was observed due to ethylene supplementation as was reported by Amoah et al., (2016). The relative expression of ERF was also selectively regulated across the spatial gradients (up-regulated at the

proximal and down-regulated at the distal section) during ethylene storage. This trend might suggest a correlation between the relative expression of ERF and phenolics metabolism. Moreover, the transition from ethylene supplementation to air storage and vice versa significantly boosted the accumulation of NSCs in the flesh as well as phenolics in the peel compared to the uninterrupted ethylene supplementation over time. A contrasting effect in sugar accumulation has been reported in sweet potato (Amoah et al., 2016) and potato (Foukaraki et al., 2014; Foukaraki et al., 2016a) as a result of the timing of ethylene supplementation. The ERF gene was upregulated at the proximal section of the root irrespective of the tissue as well as at the distal peel section as a result of truncating ethylene during storage. Air storage up-regulated the relative expression of ERF with a corresponding increase in phenolics accumulation over time. However EIN2 was significantly down regulated with a concurrent increase in NSCs and phenolics during storage. Random amplified polymorphic DNA (RAPD) has been used as a useful tool to evaluate genetic variations in Taiwanese sweet potatoes.

5.4.2 Gene expression and phytohormones cross talk during sweet potato storage

Unlike the peel, flesh ABA concentrations decreased over time except in the case of the transitions. In addition the baseline concentration of flesh ABA halved during storage under air and ethylene treatments respectively. A similar trend in flesh ABA was reported with sweet potatoes stored at 25˚C by Amoah et al. (2016) when treated with continuous ethylene and air. This suggested that ABA could have exhibited a declining response to stress signals, leading to its suppression, as a result of the abiotic conditions created by ethylene supplementation and air storage over time (Kundu and Gantait, 2017). In contrast, ethylene supplementation yielded the opposite effect on flesh ABA concentration in potato (Foukaraki et al., 2016a). As revealed by this study, the increased relative expression of ERF at the proximal section had differential influences on ABA in the flesh and peel of the sweet potato during ethylene storage. This could be related by assumption, to ERFs regulation of plant abiotic stress responses due to exogenous ethylene supplementation whilst integrating endogenous ethylene and ABA in the process (Müller and Munné-Bosch, 2015). Similarly, the decline in relative expression of EIN2 also differentially influenced ABA concentration during ethylene storage. The underlying mechanisms controlling this phenomenon are unclear. Unlike

ethylene supplementation, continuous air storage up-regulated ERF in contrast to EIN2 which corresponded to decreased and increased flesh and peel ABA concentrations respectively. Ethylene supplementation also significantly increased phaseic acid (PA) in the flesh and peel over time irrespective of spatial orientation. The increased relative expression of ERF at the proximal section of the sweet potato as opposed to the distal section further reveals the gene's differential influence in regulating PA like in ABA. Also the relative expressions of the ERF and EIN2 genes in regulating trans-ZR follow the same pattern as in ABA. Interestingly, the decline in trans-ZR could not inhibit the relative expression of the ERF gene in the proximal section of the sweet potato but did for the EIN2 gene over time. This is inconsistent with the report by Amoah et al., (2016) whose findings revealed a steady rise in the ZR content of the sweet potato cv. Covington during ethylene storage. The plant growth regulators, ABA and ZR have been linked to sprouting in sweet potato (Amoah et al., 2016). Interestingly, despite the considerable inhibition of ABA biosynthesis during continuous CA and ethylene storage, sprout suppression was successful suggesting a dual role of ethylene in its interaction with ABA and dormancy control. Increased cytokinin content as well as its sensitivity has been linked to dormancy loss in potato (Suttle, 2004). However, trans-ZR in this study declined during storage which may explain why no spouting was recorded.

5.5 Conclusions

In conclusion, ethylene supplementation during storage inhibited the accumulation of the monosaccharides, fructose and glucose with concurrent increase in sucrose. Controlled atmosphere and air storage on the other yielded the opposite effect on monosaccharides accumulation in the flesh unlike the peel of the sweet potato. The increased relative expression of ERF in the flesh during CA and air treatments corresponded with the rise in monosaccharides during storage. This study also showed a selective influence in the accumulation of the individual phenolics and phytohormones due to ethylene supplementation. Additionally, ethylene induced rise in phenolics corresponded to an increased relative expression of ERF in the proximal section of the sweet potato during storage irrespective of the root tissue. The transition from ethylene supplementation to air storage and vice versa significantly boosted the accumulation of NSCs in the flesh as well as phenolics in the peel compared to the uninterrupted

ethylene supplementation over time. Also truncating ethylene supplementation enhanced the relative expression of ERF with a corresponding phenolics accumulation in the peel, suggesting a greater natural disease resistance of the sweet potato. This study further suggested that due to the treatment transitions from ethylene to air storage, the ERF gene exhibited a reciprocating phenomenon in regulating the metabolisms of the studied plant growth hormones.

5.6 Acknowledgements

My sincere thanks and appreciation go to the Commonwealth scholarship commission for their support in providing me the opportunity to have this great experience. I would also like to thank members of the plant science group for their technical assistance.

5.7 References

Adu-Kwarteng, E., Sakyi-Dawson, E. O., Ayernor, G. S., Truong, V.D., Shih, F. F. and Daigle, K. (2014).Variability of sugars in staple-type sweet potato (*Ipomoea batatas*) cultivars: The effects of harvest time and storage. *International Journal of Food Properties*, 17(2), 410–420.

Amoah, R. S., Landahl, S., and Terry, L. A. (2016). The timing of exogenous ethylene supplementation differentially affects stored sweet potato roots. *Postharvest Biology and Technology*, 120, 92–102.

Cheema, M. U. A., Rees, D., Colgan, R. J., Taylor, M. and Westby, A. (2013). The effects of ethylene, 1-MCP and AVG on sprouting in sweet potato roots. *Postharvest Biology and Technology*, 85, 89–93.

Delate, K. M., Brecht, J. K. and Coffelt, J. A. (1990). Controlled atmosphere treatments for control of sweet potato weevil (Coleoptera: *Curculionidae*) in stored tropical sweet potatoes. *Journal of Economic Entomology*, 82, 461–465.

Duvivier, P., Hsieh, P.C., Lai, P.Y. and Charles, A.L. (2010). Retention of phenolics, carotenoids, and antioxidant activity in the Taiwanese sweet potato cv. Tainong 66 subjected to different drying conditions. *African Journal of Food, Agriculture, Nutrition and Development*, 11(10), 4413 - 4429.

Foukaraki, S. G., Cools, K., Chope, G. A., and Terry, L. A. (2014). Effect of the transition between ethylene and air storage on post- harvest quality in six UK-grown potato cultivars. *Journal of Horticultural Science and Biotechnology*, 89(6), 599–606.

Foukaraki, G., Cools, K. and Terry, L. A. (2016a). Differential effect of ethylene supplementation and inhibition on abscisic acid metabolism of potato (*Solanum tuberosum*, L.) tubers during storage, *Postharvest Biology and Technology*, 112, 87–94.

Foukaraki, G., Cools, K., Chope, G. A. and Terry, L. A. (2016b). Impact of ethylene and 1-MCP on sprouting and sugar accumulation in stored potatoes. *Postharvest Biology and Technology*, 114, 95–103.

Jung, J.-K., Lee, S.U., Kozukue, N., Levin, C. E. and Friedman, M. (2011). Distribution of phenolic compounds and antioxidative activities in parts of sweet potato (*Ipomoea batatas* L.) plants and in home processed roots. *Journal of Food Composition and Analysis*, 24(1), 29–37.

Kundu, S. and Gantait, S. (2017). Abscisic acid signal crosstalk during abiotic stress response. *Plant Gene*; 11, 61–69.

Li, X. Q. and Zhang, D. (2003). Gene expression activity and pathway selection for sucrose metabolism in developing storage root of sweet potato. *Plant and Cell Physiology*, *44*(6), 630–636.

Lin, K. H., Lai, Y. C., Li, H. C., Lo, S. F., Chen, L. F. O. and Lo, H. F. (2009). Genetic variation and its relationship to root weight in the sweet potato as revealed by RAPD analysis. *Scientia Horticulturae*, 120(1), 2–7.

Merchante, C., Alonso, J. M. and Stepanova, A. N. (2013). Ethylene signaling: Simple ligand, complex regulation. *Current Opinion in Plant Biology*, 16(5), 554–560.

Müller, M. and Munné-Bosch, S. (2015). Ethylene Response Factors: A key regulatory hub in hormone and stress signaling. *Plant Physiology*, 169(1), 32–41.

Ponniah, S. K., Thimmapuram, J., Bhide, K., Kalavacharla, V. and Manoharan, M. (2017). Comparative analysis of the root transcriptomes of cultivated sweet potato (*Ipomoea batatas*, L.) and its wild ancestor (*Ipomoea trifida*, G.). *BMC Plant Biology*, 17(1), 1 - 14

Shekhar, S., Mishra, D., Kumar, A. and Chakraborty, S. (2015), Comparative analysis of phytochemicals and nutrient availability in two contrasting cultivars of sweet potato (*Ipomoea batatas*, L.). *Food Chemistry*; 173, 957–965.

Tao, X., Gu, Y.H., Wang, H.Y., Zheng, W., Li, X., Zhao, C.W. and Zhang,Y.-Z.(2012). Digital gene expression analysis based on integrated de novo transcriptome assembly of sweet potato (*Ipomoea batatas*, L.). PLoS *ONE*, 7(4), 1–14.

6 GENERAL DISCUSSION AND IMPLICATION OF WORK

6.1 General discussion

6.1.1 The effects of CA storage conditions on changes in the postharvest physiology of sweet potato

Weight loss and sprouting are among the major physiological factors that limit the marketability of sweet potato during storage. This study has shown parallel increases in weight loss and respiration rates during ethylene supplemented CA storage and after transition to air storage which implied the creation of a stressful environment that triggered accelerated metabolic rates and greater moisture loss in the sweet potato. Truncating continuous CA treatment yielded the opposite effect as did its uninterrupted application, suggesting associated reduced metabolic activity due to depleted oxygen and increased CO_2 levels. In contrast to Rees et al., (2003) who reported a positive correlation between respiration rate and weight loss in sweet potato, this study showed no such correlation (R = 0.35 and 0.53 for Experiment 1 and 2 respectively) between the two factors during storage.

Despite the fact that weight loss and respiration rates increased during ethylene supplemented CA storage, sprout growth was inhibited. However, in spite of the success of sprout suppression during storage under CA with and without supplemental ethylene the treatments were characterized with splitting on the surface of the sweet potato which could limit its marketability of the root. A similar pattern of sprouts growth at the proximal section of sweet potato has been reported during exogenous ethylene (10 µL L^{-1}) storage (Amoah et al., 2016). Also continuous CA storage as well as the transition to and from air storage was characterized with significantly less sprout growth compared to continuous air treatment. This more or less concurred with a report by Khanbari and Thompson, (1996) who found a complete inhibition of sprouting in ware potato during CA storage at 5˚C with extended sprout suppression when stored in air afterwards. However, it was interesting to see that irrespective of the exponential increase in respiration rate as a result of truncating CA with and without supplemental ethylene, sprout development was nonetheless inhibited compared to continuous air storage.

Sprout growth has also been closely linked with changes in ABA, ZR and ethylene in potato during storage (Sonnewald and Sonnewald, 2014; Ordaz-Ortiz et al. 2015; Foukaraki et al., 2016a). The current study indicated a decline in flesh ABA concentration with concomitant rise in the cytokinin, trans-ZR, of the sweet potato during storage in Experiment 2 (Chapter 3) whereas a decline in the cytokinin was observed in Experiment 4 (Chapter 5). The implications of ABA and ZR (the major cytokinin in sweet potato) in controlling sprout growth have been reported (Amoah et al., 2016). Interestingly, even though ABA biosynthesis was considerably inhibited during continuous CA and ethylene storage, sprouting was suppressed suggesting a dual role of ethylene in its interaction with ABA and dormancy control. In contrast, CA with and without supplemental ethylene enhanced the biosynthesis of trans-zeatin riboside (trans-ZR) in the sweet potato during storage despite inhibiting sprout growth. Increased cytokinin content as well as its sensitivity has been linked to dormancy loss in potato (Suttle, 2004). However, the accelerated biosynthesis of trans-ZR in Experiment 2 did translate into increased sprouting during air storage unlike in Experiment 4 (Chapter 5) where interestingly the decreased biosynthesis of trans-ZR could have played a part in completely inhibiting sprouting. The selective influence of the storage treatments on the plant growth hormone is a possibility as sprout growth was effectively inhibited during storage under CA with and without supplemental ethylene in spite of increased trans-ZR accumulation. Also it appears that ABA could have exhibited minimal response to stress signals, leading to its suppression, as a result of the abiotic conditions created by ethylene supplementation and CA storage over time (Kundu and Gantait, 2017).

Sugar accumulation has also been linked to sprouting in sweet potato; according to Lin et al. (2011) sprouting in sweet potato correlated with sugar accumulation and β-amylase activity during storage. The fact that sprouting during storage was virtually prevented in Experiment 4 could be related to the reduced metabolic activity due to decreased trans-ZR biosynthesis. Plant growth regulators control the activation of genes that underpin the regulatory mechanisms of plant tissues. As revealed in this study, the increased relative expression of ERF at the proximal section had a differential influence on ABA in the flesh and peel of the sweet potato during ethylene storage (Experiment 4). This could be related by assumption, to the selective response of the root tissues to

ERF's regulation of abiotic stress due to ethylene supplementation whilst integrating endogenous ethylene and ABA in the process (Müller and Munné-Bosch, 2015). Also the relative expressions of the ERF and EIN2 genes in regulating trans-ZR follow the same pattern as in ABA. Interestingly, the decline in trans-ZR could not inhibit the relative expression of the ERF gene in the proximal section of the sweet potato but did for the EIN2 gene over time. Similarly, the decline in relative expression of EIN2 correlated with trans-ZR but differentially influenced ABA concentration during ethylene storage.

6.1.2 The effects of ethylene supplemental CA storage on fungal populations and potential aflatoxin production on sweet potato

The additive treatment of CA to ethylene is expected to make the process of inhibiting the adverse effects of ethylene (e.g. microbial decay) on the sweet potato more effective as was recommended by Amoah et al., (2016). However, this study suggested otherwise (Experiment 3: Chapter 4). Continuous CA treatment proved to be an effective alternative as opposed to ethylene supplemented CA in enhancing the natural disease resistance of the sweet potato (due to the pathogen-environment-host interaction) to fungal attack and/or further development and consequent decay during storage. Increased level of decay has also been reported as a result of continuous exogenous ethylene at the proximal section of sweet potato (Amoah et al., 2016). Also Lewthwaite et al. (2013) pointed out that disease susceptibility in sweet potato is further exacerbated by piercing wounds on the root surface. Tissue disruption (i.e. splitting or wounding) was noticed in this study on sweet potatoes stored under CA with and without supplemental ethylene. However, it was unclear why the splitting on the root surface during CA storage did not enhanced disease as opposed to CA supplemented with ethylene. As a means of addressing disease incidence, Afek and Orenstein (2003) reported treatment of the sweet potato (cv. 'Georgia Jet') with steam during storage for a period of five months, as an effective method in reducing decay (3% in cured roots and 14% in non-cured compared to 32% and 86% in non-treated cured and non-cured roots respectively). The effects of 1-Methylcyclopropene (1-MCP) on natural disease resistance in stored sweet potato have also been reported (Amoah and Terry, 2018).

The storage treatments in this study were ineffective in controlling *Fusarium* development but were effective in controlling *Penicillium* development over time. The identification of *Rhizopus* soft rot, caused by *Rhizopus stolinifer* and surface rot caused by *Fusarium oxysporum* as the predominant diseases on the sweet potatoes agrees with earlier reports (Ray and Ravi, 2005; Lewthwaite et al., 2013; Amoah et al., 2016; El Neshawy, 2016) that these two diseases are the most common in tropical conditions. However, infection rates have been shown to be dependent on the sweet potato cultivar, which in this study could not be established because only one cultivar was used. A report by Olaitan (2012) suggested that modified atmosphere storage using a polythene bag of 18µm thickness, can effectively control fungal disease incidence on sweet potato.

The fungal isolates identified in this study were potentially mycotoxigenic but the investigations were limited to that of the Aspergillus species (n = 24) for their potential for aflatoxins production. Increased water activity (a_w) (0.99 - 0.96) has been associated with increased production of AFB1 in maize (Abdel-Hadi et al., 2012) and corn (Astoreca et al., 2014) under tropical conditions (25 - 30˚C). It is generally accepted that AFB1 production declines with decreasing water activity. Although the water activity of the sweet potato samples was not determined, the measured relative humidity during storage (85 – 95%) gave an idea of the water activity. The sweet potatoes treated with continuous CA were all free of *Aspergillus* contamination indicating its potential effectiveness in controlling aflatoxin production during storage. Williams et al., (2014) reported effective prevention of *Aspergillus flavus* growth and aflatoxin B1 production in maize as a result of storage in a low oxygen/high CO_2 environment over a period of two months. Likewise, in this study, the diffusion of higher CO_2 levels through the sweet potato root surface during continuous CA storage may have created an unfavourable environment for the fungi to develop.

6.1.3 The effects of CA and ethylene on gene regulation of biochemical dynamics in sweet potato during storage

The decline in reducing sugars (fructose and glucose) with a concomitant rise in sucrose due to ethylene supplementation (0.001 kPa) in this study agrees with earlier reports by Cheema et al., (2013) and Amoah et al., (2016) in sweet potato. The opposite effect on the monosaccharides has been reported as a result of ethylene treatment in stored potato

(Foukaraki et al., 2016b). However, fructose accumulation in the non-ethylene treated roots in all conducted experiments concurs with Adu-Kwarteng et al. (2014) who observed a similar pattern in stored sweet potatoes under ambient conditions. The disproportionately higher levels of sucrose could be due to starch hydrolysis triggered by the activation of sucrose phosphate synthase in the root tissues of the sweet potato (Li and Zhang, 2003; Tao et al., 2012; Ponniah et al., 2017). Unlike the monosaccharides in the peel, this study revealed enhanced accumulation of the NSCs over time as a result of air and CA treatments. The tight regulation of genes has been associated with reduced starch degradation in sweet potato (Shekhar et al., 2015). The results in this study (Experiment 4) also showed that the storage treatments largely enhanced accumulation of phenolics over time except for peel chlorogenic acid. The treatments also showed a selective influence in the accumulation of the individual phenolics due to ethylene supplementation. A similar report had been made (Amoah et al., 2016) on sweet potato stored under continuous exogenous ethylene at 25˚C. However, the ERF gene expression in the proximal section of the sweet potato was up-regulated during ethylene storage as opposed to the distal section where it was significantly down-regulated irrespective of the root tissue over time. This trend might suggest a correlation between the relative expression of ERF and phenolics metabolism. Air storage up-regulated the relative expression of ERF irrespective of the sweet potato root tissue and spatial orientation which corresponded to the increased phenolics accumulation over time except peel chlorogenic acid. Air storage followed by CA was effective in lowering NSCs in the flesh of the sweet potato, so was continuous CA, compared to air storage. This could be attributed to the reduced metabolism of the NSCs due to depleted O_2 levels. Likewise flesh Iso-A and chlorogenic acids followed a similar pattern after transition from air to CA storage. Although CA storage with supplemental ethylene accelerated the accumulation of the NSCs, the metabolisms of the studied phenolics in the peel were inhibited. The disproportionately higher contents of phenolics in the peel than in the flesh of the sweet potato could be related to their facilitation of wound healing and also their likelihood to aiding resistance against pathogens (Duvivier et al., 2010; Jung et al., 2011). The transitions from CA with and without supplemental ethylene to air storage boosted all phenolics in the flesh but only Iso-B and Iso-C in the peel over time. Coincidentally, sprout proliferation was observed

after this transition compared to continuous air storage. Amoah et al. (2016) have reported a similar possibility during the break in dormancy of stored sweet potatoes treated with continuous ethylene and air. The increased phenolic content during continuous CA storage in this study also suggested a link to the reduced disease incidence as was observed in the Experiment 3 (chapter four – section 4.3.1). Aiding natural resistance against pathogens by phenolics has also been reported in potato (Pihlanto, 2011). The application of 1-MCP prior to ethylene storage and that of the timing of ethylene supplementation have also been linked to sugar accumulation in potato (Foukaraki et al., 2016b). Moreover, the transition from ethylene supplementation to air storage and vice versa significantly boosted the accumulation of NSCs in the flesh as well as phenolics in the peel compared to the uninterrupted ethylene supplementation over time. A contrasting effect in sugar accumulation has been reported in sweet potato (Amoah et al., 2016; Amoah and Terry, 2018) and potato (Foukaraki et al., 2014; Foukaraki et al., 2016a) as a result of the timing of ethylene supplementation.

6.2 Implications of the study on sweet potato storage

This study demonstrated that controlled atmosphere and ethylene could be a viable option to maintaining the postharvest quality of sweet potato during storage. The additive treatment of CA to exogenous ethylene showed to be inefficient in minimizing weight loss as well as splitting on the surface of the sweet potato which could limit the marketability of the root. The transition from continuous CA with and without supplemented ethylene to air storage and vice versa were effective in reducing sprouting which would translate into economic returns on the use of this technology in the tropical regions like in Sierra Leone. In as much as the treatments were successful in supressing sprouting, the content of sugars was increased which undermine the positive aspects of continuous ethylene, continuous CA and ethylene supplemented CA during storage. This dynamic in effect would also limit its consumer appeal especially for processing into diverse local products in Sierra Leone and West Africa.

Continuous CA storage was very effective in mitigating disease development on the sweet potato as much as reducing the potential for aflatoxin production. Therefore the health hazard posed as a result of fungal activity on the sweet potato is highly unlikely

during CA storage. This is also a potentially effective control strategy for mycotoxin development on sweet potato.

Controlled atmosphere storage is an expensive means of storage especially under tropical conditions. However this study has provided an insight into the possibility of utilising this technology under relative higher temperature situations. With the proposed commercialization and industrialization of the agricultural sector in Sierra Leone, there is the potential of CA storage to be introduced into the agricultural supply chain in the country. An integrated approach of other crop storage management strategies with CA storage could be effective in minimizing the very huge amount of postharvest losses taking place in Sierra Leone. With regards to sourcing the needed gases, that can be tackled through bio gas generation using local agricultural plant products especially with the introduction of the Innovation platform approach to farming practices in the country.

6.3 References

Adu-Kwarteng, E., Sakyi-Dawson, E. O., Ayernor, G. S., Truong, V.D., Shih, F. F. and Daigle, K. (2014).Variability of sugars in staple-type sweet potato (*Ipomoea batatas*) cultivars: The effects of harvest time and storage. *International Journal of Food Properties*, 17(2), 410–420.

Abdel-Hadi, A., Schmidt-Heydt, M., Parra, R., Geisen, R. and Magan, N. (2012). A systems approach to model the relationship between aflatoxin gene cluster expression, environmental factors, growth and toxin production by *Aspergillus flavus*. *Journal of the Royal Society Interface*, 9, 757–767.

Afek, U., Orenstein, J., and Nuriel, E. (2000). Using HPP (hydrogen peroxide plus) to inhibit potato sprouting during storage. *American Journal of Potato Research*, 77(1), 63–65.

Amoah, R. S., Landahl, S., and Terry, L. A. (2016). The timing of exogenous ethylene supplementation differentially affects stored sweet potato roots. *Postharvest Biology and Technology*, 120, 92–102

Amoah, R.S. and Terry, L.A. (2018). 1-Methylcyclopropene (1-MCP) effects on natural disease resistance in stored sweet potato. *Journal of the Science of Food and Agriculture*, in Press

Astoreca, A., Vaamonde, G., Dalcero, A., Marin, S. and Ramos, A. (2014). Abiotic factors and their interactions influence on the co-production of aflatoxin B(1) and cyclopiazonic acid by *Aspergillus flavus* isolated from corn. *Food Microbiology*, 38, 276–83.

Cheema, M. U. A., Rees, D., Colgan, R. J., Taylor, M. and Westby, A. (2013). The effects of ethylene, 1-MCP and AVG on sprouting in sweet potato roots. *Postharvest Biology and Technology*, 85, 89–93.

Duvivier, P., Hsieh, P.C., Lai, P.Y. and Charles, A.L. (2010). Retention of phenolics, carotenoids, and antioxidant activity in the Taiwanese sweet potato cv. Tainong 66 subjected to different drying conditions. *African Journal of Food, Agriculture, Nutrition and Development*, 11(10), 4413 - 4429.

El Neshawy, S. M. (2016). Management of the whole process of fresh Egyptian sweet potato prepared for export against soft rot. *Acta Horticulturae*, 1144, 417-422.

Foukaraki, S. G., Cools, K., Chope, G. A., and Terry, L. A. (2014). Effect of the transition between ethylene and air storage on post- harvest quality in six UK-grown potato cultivars. *Journal of Horticultural Science and Biotechnology*, 89(6), 599–606.

Foukaraki, G., Cools, K. and Terry, L. A. (2016a). Differential effect of ethylene supplementation and inhibition on abscisic acid metabolism of potato (*Solanum tuberosum*, L.) tubers during storage, *Postharvest Biology and Technology*, 112, 87–94.

Foukaraki, G., Cools, K., Chope, G. A. and Terry, L. A. (2016b). Impact of ethylene and 1-MCP on sprouting and sugar accumulation in stored potatoes. *Postharvest Biology and Technology*, 114, 95–103.

Jung, J.-K., Lee, S.U., Kozukue, N., Levin, C. E. and Friedman, M. (2011). Distribution of phenolic compounds and antioxidative activities in parts of sweet potato

(*Ipomoea batatas* L.) plants and in home processed roots. *Journal of Food Composition and Analysis*, 24(1), 29–37.

Kundu, S. and Gantait, S. (2017). Abscisic acid signal crosstalk during abiotic stress response. *Plant Gene*; 11, 61–69.

Lewthwaite, S. L., Wright, P. J. and Triggs, C. M. (2013). Sweet Potato Cultivar Susceptibility to Postharvest Soft Rot Caused by *Rhizopus stolonifer*. *New Zealand Plant Protection*, 66, 223–228.

Li, X. Q. and Zhang, D. (2003). Gene expression activity and pathway selection for sucrose metabolism in developing storage root of sweet potato. *Plant and Cell Physiology*, *44*(6), 630–636.

Olaitan, O. O. (2012). Bio-deterioration of sweet potato (*Ipomoea batatas* L.) in storage, inoculation-induced quality changes and control by modified atmosphere. *Journal of Applied Science and Environmental Management*, 16(2), 189–193.

Ordaz-ortiz, J., Foukaraki, S., and Terry, L. A. (2015). Assessing temporal flux of plant hormones in stored processing potatoes using high definition accurate mass spectrometry. *Horticultural Research*, 2, 1–8.

Pihlanto, A. (2011). 'Potato and Other Root Crops' in Terry, L.A. (ed) *Health-promoting Properties of Fruits and Vegetables*. CABI, Wallingford, p. 218-233.

Ponniah, S. K., Thimmapuram, J., Bhide, K., Kalavacharla, V. and Manoharan, M. (2017). Comparative analysis of the root transcriptomes of cultivated sweet potato (*Ipomoea batatas*, L.) and its wild ancestor (*Ipomoea trifida*, G.). *BMC Plant Biology*, 17(1), 1 – 14.

Ray, R. C. and Ravi, V. (2005). Postharvest spoilage of sweetpotato in tropics and control measures. *Critical Reviews in Food Science and Nutrition*, 45(7–8), 623–644.

Rees, D., van Oirschot, Q. E. ., Amour, R., Rwiza, E., Kapinga, R., and Carey, T. (2003). Cultivar variation in keeping quality of sweet potatoes. *Postharvest Biology and Technology*, 28(2), 313–325.

Shekhar, S., Mishra, D., Kumar, A. and Chakraborty, S. (2015), Comparative analysis of phytochemicals and nutrient availability in two contrasting cultivars of sweet potato (*Ipomoea batatas*, L.). *Food Chemistry*; 173, 957–965.

Sonnewald, S. and Sonnewald, U. (2014). Regulation of potato tuber sprouting, *Planta*, 239, 27–38

Suttle, J.C. (2004).Physiological regulation of potato tuber dormancy, *American Journal of Potato Research*, 81, 253–262.

Tao, X., Gu, Y.H., Wang, H.Y., Zheng, W., Li, X., Zhao, C.W. and Zhang,Y.-Z.(2012). Digital gene expression analysis based on integrated de novo transcriptome assembly of sweet potato (*Ipomoea batatas*, L.). PLoS *ONE*, 7(4), 1–14.

Williams, S. B., Baributsa, D. and Woloshuk, C. (2014). Assessing Purdue Improved Crop Storage (PICS) bags to mitigate fungal growth and aflatoxin contamination. *Journal of Stored Products Research*, 59, 190–196.

7 CONCLUSION AND FURTHER RESEARCH

7.1 Conclusions

The overall findings of this study demonstrated that controlled atmosphere and ethylene could be a viable option to maintaining the postharvest quality of sweet potato during storage. The specific conclusions with regards to the objectives are as follows:

Objective 1. ***To determine the effects of respiratory gases (CO_2 and O_2) on the respiration rates, weight loss and spatial effects on dormancy and sprouting of sweet potato during storage*****.**

- The additive treatment of CA to exogenous ethylene showed to be inefficient in minimizing weight loss as well as the respiration rates of the sweet potato during storage. Continuous treatment of CA with and without ethylene was characterized with splitting on the surface of the sweet potato which could limit the marketability of the root. (Chapter 3)

- Continuous CA with and without supplemented ethylene as well as their transitions to air storage and vice versa was characterized with significantly less sprouts growth compared to continuous air treatment. (Chapter 3)

- Truncating the ethylene supplemented CA boosted sprouting compared to its uninterrupted application which makes it a non-viable option in terms of minimizing sprout growth on sweet potato. (Chapter 3)

Objective 2. ***To determine the spatial distribution of non-structural carbohydrates, polyphenols and plant growth hormones of sweet potato during storage in CA and ethylene conditions.***

- In as much as the treatments were successful in supressing sprouting, the metabolism of phenolics and sugars was boosted which undermine the positive aspects of continuous ethylene, continuous CA and ethylene supplemental CA during storage. The accelerated synthesis of these metabolites was as a result of the stressful environment created during storage which translated into increased respiratory energy and water loss in the sweet potato.(Chapter 3 and 5)

- Despite the considerable inhibition of ABA biosynthesis during continuous CA and ethylene storage, sprout suppression was successful suggesting a dual role of ethylene in its interaction with ABA and dormancy control.(Chapter 3 and 5)

- The complete inhibition of sprouting during storage (Experiment 4) was attributed to decreased biosynthesis of trans-zeatin riboside (trans-ZR) in the sweet potato due to ethylene supplementation.(Chapter 3 and 5)

- The storage treatments differentially influenced the plant growth hormones in effecting changes in the physiology of the stored sweet potato.(Chapter 3 and 5)

Objective 3. ***To determine the effects of storage of sweet potato in CA and ethylene on potential fungal and associated aflatoxin development across the different spatial gradients of the root***

- Continuous CA Storage was effective in mitigating disease development on the sweet potato. The storage method was also effective in controlling *Penicillium* development but not that of *Fusarium*. (Chapter 4)

- Major diseases identified on the sweet potato were *Fusarium* surface rot as well as *Rhizopus* soft rot predominantly at the proximal and distal sections. The diseased roots were mainly those stored under ethylene supplemented CA. (Chapter 4)

- Aflatoxin G1 was the most predominant potentially produced aflatoxin on the sweet potato but continuous CA was very effective in controlling the potential contamination of the studied aflatoxins during storage. (Chapter 4)

- Despite the fact that in-vitro analyses was not done, the results could be useful in designing effective control strategies for aflatoxins contamination, hence minimising the health risks associated with fungal activity during storage. (Chapter 4)

Objective 4. ***To investigate the molecular and metabolic mechanisms associated with the post-harvest quality of sweet potato during CA and ethylene storage.***

- The increased relative expression of the ERF and EIN2 genes at the proximal section had a differential influence in regulating ABA and trans-ZR biosynthesis of the sweet potato during ethylene storage. (Chapter 5)

- The ERF and EIN2 genes had a reciprocating regulatory pattern in the tissue metabolism of the sweet potato during storage. The ERF was more expressed towards the end of storage whilst EIN2 was more expressed at the beginning. (Chapter 5)

- The increased relative expression of the ERF gene in the flesh at the proximal section during ethylene storage, did not translate in a concurrent increase in the reducing sugars. The mechanisms surrounding this relationship are not clear.
- The transition from continuous supplemental ethylene to air storage enhanced the relative expression of the ERF gene in the peel tissue of the sweet potato which also corresponded to increased phenolics accumulation in spite of spatial difference.

The current study has shown that continuous CA storage of sweet potato provides better outcome in terms of enhancing its quality over time compared to that supplemented with exogenous ethylene and also that of continuous exogenous ethylene. Despite the fact that splitting was observed after nine weeks of storage under continuous CA treatment, mitigating fungal infection and disease and also inhibiting the potential of mycotoxin contamination, makes the treatment a viable option as opposed to the others.

7.2 Further research suggestions

- In vitro experiments must be carried out to ascertain the actual growth rate of fungi and the actual production of aflatoxins and other related mycotoxins.
- The biochemical basis surrounding spatial influence on fungal growth and is not fully explained in this report which warrants further investigation.
- Physiological data (e.g. respiration rates) need to be linked with changing expression of genes
- The inter connection between the metabolites (sugars and phenolics) and the studied genes need further elucidation in order to establish the actual biological mechanisms surrounding their relationship
- More sweet potato cultivars need to be tested under CA using different storage temperatures and also different CA regimes to ascertain the temperature-CA mix that best suits the sweet potato.

APPENDICES

Appendix A – Chapter three: The effects of supplemental ethylene and controlled atmosphere on the physiology and biochemistry of stored sweet potatoes

Table A-1 ANOVA table - the effects of ethylene supplemental CA on weight loss (%) of sweet potato during storage (Experiment 1: 2014-15).

Effect	SS	d.f.	MS	F	p
Intercept	460.9130	1	460.9130	282.7639	0.000000
Baseline	326.4801	1	326.4801	200.2911	0.000000
Outturn(Baseline)	160.6087	1	160.6087	98.5313	0.000000
Treatment(Baseline)	170.8888	4	42.7222	26.2095	0.000000
OutturnTreatment(Baseline)	23.2611	2	11.6305	7.1352	0.005227
Error	29.3405	18	1.6300		

Table A-2 ANOVA table - the effects of supplemental CA on the rate of respiration of sweet potato during storage (Experiment 1: 2014-15).

Effect	SS	df	MS	F	p
Intercept	0.000000	1	0.000000	1610.682	0.000000
Baseline	0.000000	1	0.000000	144.750	0.000000
Outturn(Baseline)	0.000000	1	0.000000	428.298	0.000000
Treatment(Baseline)	0.000000	2	0.000000	4.352	0.035734
OutturnTreatment(Baseline)	0.000000	2	0.000000	4.353	0.035719
Error	0.000000	13	0.000000		

Table A-3 ANOVA table - the effects of ethylene supplemental CA on weight loss (%) of sweet potato during storage (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	651.4884	1	651.4884	60.64876	0.000000
Baseline	242.4873	1	242.4873	22.57378	0.000014
storage time (baseline)	356.5947	4	89.1487	8.29908	0.000024
treatment(baseline)	235.3251	10	23.5325	2.19070	0.031385
storage time treatment(baseline)	106.9294	13	8.2253	0.76572	0.691450
Error	612.2935	57	10.7420		

Table A-4 ANOVA table - the effects of ethylene supplemental CA on the respiration rates of sweet potato during storage (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	0.000000	1	0.000000	996.6635	0.000000
Trans	0.000000	2	0.000000	208.0756	0.000000
Time (weeks)(Trans)	0.000000	4	0.000000	0.2340	0.917870
Treatment(Trans)	0.000000	8	0.000000	155.7525	0.000000
Time (weeks)Treatment(Trans)	0.000000	12	0.000000	2.1594	0.029451
Error	0.000000	49	0.000000		

Table A-5 ANOVA table - the effects of ethylene supplemental CA on sprout growth during storage of sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	32.89695	1	32.89695	648.9661	0.000000
Trans	3.95107	1	3.95107	77.9437	0.000000
Section(Trans)	2.74344	2	1.37172	27.0603	0.000000
Time (weeks)(Trans)	12.10869	5	2.42174	47.7742	0.000000
Treatment(Trans)	3.63178	5	0.72636	14.3290	0.000000
Time (weeks)Treatment(Trans)	2.32662	11	0.21151	4.1725	0.000065
Time (weeks)Section(Trans)	0.85692	5	0.17138	3.3809	0.007849
TreatmentSection(Trans)	0.30165	5	0.06033	1.1901	0.321016
Time(weeks)TreatmentSection(Trans)	0.15517	11	0.01411	0.2783	0.988356
Error	4.25807	84	0.05069		

Table A-6 ANOVA table - the effects of ethylene supplemental CA on flesh fructose accumulation in stored sweet potato (Experiment 1: 2014-15)

Effect	SS	df	MS	F	p
Intercept	354775.3	1	354775.3	518.0475	0.000000
Baseline	6894.8	1	6894.8	10.0679	0.003646
Outturn(Baseline)	11499.9	1	11499.9	16.7923	0.000323
Treatment(Baseline)	814.8	2	407.4	0.5949	0.558452
Section(Baseline)	3820.9	2	1910.5	2.7897	0.078560
OutturnTreatment(Baseline)	2376.0	2	1188.0	1.7347	0.194880
OutturnSection(Baseline)	226.5	1	226.5	0.3307	0.569845
TreatmentSection(Baseline)	549.8	2	274.9	0.4014	0.673186
OutturnTreatmentSection(Baseline)	125.1	2	62.5	0.0913	0.912999
Error	19175.3	28	684.8		

Table A-7 ANOVA table - the effects of ethylene supplemental CA on flesh glucose accumulation in stored sweet potato (Experiment 1: 2014-15)

Effect	SS	df	MS	F	p
Intercept	91156.38	1	91156.38	413.5282	0.000000
Baseline	2065.75	1	2065.75	9.3712	0.004825
Outturn(Baseline)	3145.36	1	3145.36	14.2688	0.000761
Treatment(Baseline)	1248.43	2	624.22	2.8317	0.075855
Section(Baseline)	2812.37	2	1406.19	6.3791	0.005214
OutturnTreatment(Baseline)	542.13	2	271.07	1.2297	0.307695
OutturnSection(Baseline)	349.22	1	349.22	1.5842	0.218546
TreatmentSection(Baseline)	163.90	2	81.95	0.3718	0.692872
OutturnTreatmentSection(Baseline)	75.38	2	37.69	0.1710	0.843705
Error	6172.20	28	220.44		

Table A-8 ANOVA table - the effects of ethylene supplemental CA on flesh sucrose accumulation in stored sweet potato (Experiment 1: 2014-15)

Effect	SS	df	MS	F	p
Intercept	741006.3	1	741006.3	1158.657	0.000000
Baseline	12821.4	1	12821.4	20.048	0.000116
Outturn(Baseline)	20929.9	1	20929.9	32.727	0.000004
Treatment(Baseline)	28903.7	2	14451.8	22.597	0.000001
Section(Baseline)	4390.1	2	2195.0	3.432	0.046436
OutturnTreatment(Baseline)	4618.0	2	2309.0	3.610	0.040274
OutturnSection(Baseline)	155.9	1	155.9	0.244	0.625344
TreatmentSection(Baseline)	4965.5	2	2482.7	3.882	0.032505
OutturnTreatmentSection(Baseline)	270.1	2	135.1	0.211	0.810908
Error	17907.1	28	639.5		

Table A-9 ANOVA table - the effects of ethylene supplemental CA on peel fructose accumulation in stored sweet potato (Experiment 1: 2014-15)

Effect	SS	df	MS	F	p
Intercept	16323.32	1	16323.32	250.7237	0.000000
Baseline	167.49	1	167.49	2.5726	0.120811
Outturn(Baseline)	190.09	1	190.09	2.9197	0.099417
Treatment(Baseline)	11.55	2	5.78	0.0887	0.915366
Section(Baseline)	25.29	2	12.65	0.1942	0.824642
OutturnTreatment(Baseline)	52.00	2	26.00	0.3993	0.674811
OutturnSection(Baseline)	49.77	1	49.77	0.7645	0.389926
TreatmentSection(Baseline)	12.20	2	6.10	0.0937	0.910840
OutturnTreatmentSection(Baseline)	45.78	2	22.89	0.3516	0.706878
Error	1692.73	26	65.10		

Table A-10 ANOVA table - the effects of ethylene supplemental CA on peel glucose accumulation in stored sweet potato (Experiment 1: 2014-15)

Effect	SS	df	MS	F	p
Intercept	7041.226	1	7041.226	395.6556	0.000000
Baseline	51.796	1	51.796	2.9105	0.099926
Outturn(Baseline)	22.199	1	22.199	1.2474	0.274272
Treatment(Baseline)	28.514	2	14.257	0.8011	0.459601
Section(Baseline)	12.079	2	6.039	0.3394	0.715335
OutturnTreatment(Baseline)	17.822	2	8.911	0.5007	0.611819
OutturnSection(Baseline)	14.587	1	14.587	0.8196	0.373596
TreatmentSection(Baseline)	6.146	2	3.073	0.1727	0.842364
OutturnTreatmentSection(Baseline)	4.797	2	2.398	0.1348	0.874522
Error	462.705	26	17.796		

Table A-11 ANOVA table - the effects of ethylene supplemental CA on peel sucrose accumulation in stored sweet potato (Experiment 1: 2014-15)

Effect	SS	df	MS	F	p
Intercept	781205.8	1	781205.8	1316.429	0.000000
Baseline	985.1	1	985.1	1.660	0.208544
Outturn(Baseline)	6560.4	1	6560.4	11.055	0.002554
Treatment(Baseline)	101618.8	2	50809.4	85.620	0.000000
Section(Baseline)	1144.5	2	572.3	0.964	0.393985
OutturnTreatment(Baseline)	532.1	2	266.0	0.448	0.643373
OutturnSection(Baseline)	0.4	1	0.4	0.001	0.978611
TreatmentSection(Baseline)	967.5	2	483.8	0.815	0.453148
OutturnTreatmentSection(Baseline)	867.5	2	433.7	0.731	0.490757
Error	16022.6	27	593.4		

Table A-12 ANOVA table - the effects of ethylene supplemental CA on flesh fructose accumulation in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	36459.51	1	36459.51	1993.209	0.000000
Trans	4612.69	2	2306.35	126.086	0.000000
Storage weeks(Trans)	1071.83	2	535.92	29.298	0.000000
Treatment(Trans)	8119.89	8	1014.99	55.488	0.000000
Section(Trans)	120.84	3	40.28	2.202	0.094340
Storage weeksTreatment(Trans)	2892.99	8	361.62	19.770	0.000000
Storage weeksSection(Trans)	75.32	2	37.66	2.059	0.134397
TreatmentSection(Trans)	98.11	8	12.26	0.670	0.715923
Storage weeksTreatmentSection(Trans)	186.95	8	23.37	1.278	0.267214
Error	1445.06	79	18.29		

Table A-13 ANOVA table - the effects of ethylene supplemental CA on flesh glucose accumulation in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	75836.33	1	75836.33	2960.311	0.000000
Trans	315.28	2	157.64	6.154	0.003207
Storage weeks(Trans)	383.98	2	191.99	7.494	0.001012
Treatment(Trans)	2059.05	8	257.38	10.047	0.000000
Section(Trans)	74.26	3	24.75	0.966	0.412634
Storage weeksTreatment(Trans)	2375.22	8	296.90	11.590	0.000000
Storage weeksSection(Trans)	37.40	2	18.70	0.730	0.484915
TreatmentSection(Trans)	347.52	8	43.44	1.696	0.111347
Storage weeksTreatmentSection(Trans)	379.16	8	47.39	1.850	0.078999
Error	2151.89	84	25.62		

Table A-14 ANOVA table - the effects of ethylene supplemental CA on flesh sucrose accumulation in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	4452707	1	4452707	8563.594	0.000000
Trans	6433	2	3217	6.186	0.003116
Storage weeks(Trans)	6324	2	3162	6.081	0.003415
Treatment(Trans)	24747	8	3093	5.949	0.000005
Section(Trans)	1340	3	447	0.859	0.465595
Storage weeksTreatment(Trans)	13347	8	1668	3.209	0.003148
Storage weeksSection(Trans)	472	2	236	0.454	0.636571
TreatmentSection(Trans)	25066	8	3133	6.026	0.000004
Storage weeksTreatmentSection(Trans)	23232	8	2904	5.585	0.000011
Error	43676	84	520		

Table A-15 ANOVA table - the effects of ethylene supplemental CA on peel fructose accumulation in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	8587.777	1	8587.777	7300.828	0.000000
Trans	394.502	2	197.251	167.691	0.000000
Storage weeks(Trans)	1623.246	2	811.623	689.995	0.000000
Treatment(Trans)	952.265	8	119.033	101.195	0.000000
Section(Trans)	15.366	3	5.122	4.354	0.006808
Storage weeksTreatment(Trans)	802.103	8	100.263	85.238	0.000000
Storage weeksSection(Trans)	0.266	2	0.133	0.113	0.893170
TreatmentSection(Trans)	22.388	8	2.798	2.379	0.023623
Storage weeksTreatmentSection(Trans)	5.466	8	0.683	0.581	0.790795
Error	94.102	80	1.176		

Table A-16 ANOVA table - the effects of ethylene supplemental CA on peel glucose accumulation in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	26154.50	1	26154.50	8795.921	0.000000
Trans	175.31	2	87.65	29.478	0.000000
Storage weeks(Trans)	1149.05	2	574.53	193.217	0.000000
Treatment(Trans)	47.53	8	5.94	1.998	0.056454
Section(Trans)	33.98	3	11.33	3.810	0.012988
Storage weeksTreatment(Trans)	24.43	8	3.05	1.027	0.422485
Storage weeksSection(Trans)	5.23	2	2.62	0.880	0.418726
TreatmentSection(Trans)	70.35	8	8.79	2.957	0.005785
Storage weeksTreatmentSection(Trans)	39.03	8	4.88	1.641	0.125474
Error	249.77	84	2.97		

Table A-17 ANOVA table - the effects of ethylene supplemental CA on peel sucrose accumulation in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	2633401	1	2633401	6208.324	0.000000
Trans	31325	2	15662	36.925	0.000000
Storage weeks(Trans)	4346	2	2173	5.122	0.007959
Treatment(Trans)	27297	8	3412	8.044	0.000000
Section(Trans)	8118	3	2706	6.379	0.000601
Storage weeksTreatment(Trans)	4210	8	526	1.241	0.286029
Storage weeksSection(Trans)	202	2	101	0.238	0.788730
TreatmentSection(Trans)	4814	8	602	1.419	0.200709
Storage weeksTreatmentSection(Trans)	1351	8	169	0.398	0.918685
Error	35631	84	424		

Table A-18 ANOVA table - the effects of ethylene supplemented CA on flesh chlorogenic acid in stored sweet potato (Experiment 1: 2014-15)

Effect	SS	df	MS	F	p
Intercept	4682797	1	4682797	871.6026	0.000000
Baseline	19578	1	19578	3.6440	0.066569
Storage Weeks(Baseline)	148362	1	148362	27.6144	0.000014
Treatment(Baseline)	174438	2	87219	16.2340	0.000021
Location(Baseline)	5884	2	2942	0.5476	0.584430
Storage WeeksTreatment(Baseline)	46102	2	23051	4.2905	0.023694
Storage WeeksLocation(Baseline)	9713	1	9713	1.8079	0.189547
TreatmentLocation(Baseline)	23902	2	11951	2.2244	0.126887
Storage WeeksTreatmentLocation(Baseline)	10550	2	5275	0.9819	0.387143
Error	150434	28	5373		

Table A-19 ANOVA table - the effects of ethylene supplemented CA on flesh Isochlorogenic acid A in stored sweet potato (Experiment 1: 2014-15)

Effect	SS	df	MS	F	p
Intercept	3256062	1	3256062	533.1145	0.000000
Baseline	324768	1	324768	53.1743	0.000000
Storage Weeks(Baseline)	1972	1	1972	0.3229	0.574416
Treatment(Baseline)	49996	2	24998	4.0930	0.027584
Location(Baseline)	17581	2	8790	1.4393	0.254111
Storage WeeksTreatment(Baseline)	15766	2	7883	1.2907	0.290940
Storage WeeksLocation(Baseline)	1162	1	1162	0.1902	0.666066
TreatmentLocation(Baseline)	46783	2	23391	3.8299	0.033862
Storage WeeksTreatmentLocation(Baseline)	2426	2	1213	0.1986	0.821003
Error	171013	28	6108		

Table A-20 ANOVA table - the effects of ethylene supplemented CA on flesh Isochlorogenic acid B in stored sweet potato (Experiment 1: 2014-15)

Effect	SS	df	MS	F	p
Intercept	269724.1	1	269724.1	168.2466	0.000000
Baseline	8745.0	1	8745.0	5.4549	0.026904
Storage Weeks(Baseline)	2127.8	1	2127.8	1.3273	0.259031
Treatment(Baseline)	2137.5	2	1068.7	0.6667	0.521385
Location(Baseline)	1527.5	2	763.8	0.4764	0.625945
Storage WeeksTreatment(Baseline)	12053.2	2	6026.6	3.7592	0.035798
Storage WeeksLocation(Baseline)	66.4	1	66.4	0.0414	0.840215
TreatmentLocation(Baseline)	3316.0	2	1658.0	1.0342	0.368694
Storage WeeksTreatmentLocation(Baseline)	817.6	2	408.8	0.2550	0.776698
Error	44888.1	28	1603.1		

Table A-21 ANOVA table - the effects of ethylene supplemented CA on flesh Isochlorogenic acid B in stored sweet potato (Experiment 1: 2014-15)

Effect	SS	df	MS	F	p
Intercept	364.2979	1	364.2979	17.93994	0.000237
Baseline	131.4922	1	131.4922	6.47536	0.016968
Storage Weeks(Baseline)	145.6491	1	145.6491	7.17253	0.012444
Treatment(Baseline)	532.7589	2	266.3795	13.11792	0.000105
Location(Baseline)	43.1599	2	21.5800	1.06271	0.359531
Storage WeeksTreatment(Baseline)	31.2048	2	15.6024	0.76835	0.473655
Storage WeeksLocation(Baseline)	64.3313	1	64.3313	3.16801	0.086353
TreatmentLocation(Baseline)	6.1398	2	3.0699	0.15118	0.860417
Storage WeeksTreatmentLocation(Baseline)	0.6709	2	0.3354	0.01652	0.983627
Error	548.2763	27	20.3065		

Table A-22 ANOVA table - the effects of ethylene supplemented CA on peel chlorogenic acid in stored sweet potato (Experiment 1: 2014-15)

Effect	SS	df	MS	F	p
Intercept	176455409	1	176455409	763.0266	0.000000
Baseline	183248	1	183248	0.7924	0.380961
Storage Weeks(Baseline)	344937	1	344937	1.4916	0.232157
Treatment(Baseline)	767092	2	383546	1.6585	0.208584
Location(Baseline)	304509	2	152254	0.6584	0.525521
Storage WeeksTreatment(Baseline)	392392	2	196196	0.8484	0.438815
Storage WeeksLocation(Baseline)	5673	1	5673	0.0245	0.876670
TreatmentLocation(Baseline)	309947	2	154974	0.6701	0.519655
Storage WeeksTreatmentLocation(Baseline)	135174	2	67587	0.2923	0.748825
Error	6475202	28	231257		

Table A-23 ANOVA table - the effects of ethylene supplemented CA on peel Isochlorogenic acid A in stored sweet potato (Experiment 1: 2014-15)

Effect	SS	df	MS	F	p
Intercept	227374657	1	227374657	1012.853	0.000000
Baseline	787174	1	787174	3.507	0.071605
Storage Weeks(Baseline)	849207	1	849207	3.783	0.061886
Treatment(Baseline)	4101002	2	2050501	9.134	0.000884
Location(Baseline)	725166	2	362583	1.615	0.216844
Storage WeeksTreatment(Baseline)	2215775	2	1107888	4.935	0.014589
Storage WeeksLocation(Baseline)	124818	1	124818	0.556	0.462087
TreatmentLocation(Baseline)	106377	2	53188	0.237	0.790612
Storage WeeksTreatmentLocation(Baseline)	318330	2	159165	0.709	0.500754
Error	6285698	28	224489		

Table A-24 ANOVA table - the effects of ethylene supplemented CA on peel Isochlorogenic acid B in stored sweet potato (Experiment 1: 2014-15)

Effect	SS	df	MS	F	p
Intercept	29598062	1	29598062	285.3137	0.000000
Baseline	6640	1	6640	0.0640	0.802113
Storage Weeks(Baseline)	2129207	1	2129207	20.5247	0.000100
Treatment(Baseline)	1263062	2	631531	6.0877	0.006379
Location(Baseline)	249540	2	124770	1.2027	0.315420
Storage WeeksTreatment(Baseline)	1470785	2	735392	7.0889	0.003229
Storage WeeksLocation(Baseline)	190143	1	190143	1.8329	0.186612
TreatmentLocation(Baseline)	35519	2	17759	0.1712	0.843534
Storage WeeksTreatmentLocation(Baseline)	148890	2	74445	0.7176	0.496667
Error	2904682	28	103739		

Table A-25 ANOVA table - the effects of ethylene supplemented CA on peel Isochlorogenic acid C in stored sweet potato (Experiment 1: 2014-15)

Effect	SS	df	MS	F	p
Intercept	2885417	1	2885417	298.1204	0.000000
Baseline	115693	1	115693	11.9534	0.001761
Storage Weeks(Baseline)	401141	1	401141	41.4458	0.000001
Treatment(Baseline)	52609	2	26304	2.7178	0.083425
Location(Baseline)	13725	2	6862	0.7090	0.500745
Storage WeeksTreatment(Baseline)	111400	2	55700	5.7549	0.008060
Storage WeeksLocation(Baseline)	16793	1	16793	1.7350	0.198447
TreatmentLocation(Baseline)	2743	2	1372	0.1417	0.868496
Storage WeeksTreatmentLocation(Baseline)	38565	2	19282	1.9922	0.155260
Error	271003	28	9679		

Table A-26 ANOVA table - the effects of ethylene supplemented CA on peel caffeic acid in stored sweet potato (Experiment 1: 2014-15)

Effect	SS	df	MS	F	p
Intercept	1491917	1	1491917	311.0788	0.000000
Baseline	59232	1	59232	12.3504	0.001574
Storage Weeks(Baseline)	34931	1	34931	7.2834	0.011856
Treatment(Baseline)	259545	2	129773	27.0589	0.000000
Location(Baseline)	3390	2	1695	0.3534	0.705470
Storage WeeksTreatment(Baseline)	9322	2	4661	0.9719	0.391207
Storage WeeksLocation(Baseline)	1580	1	1580	0.3295	0.570680
TreatmentLocation(Baseline)	16737	2	8368	1.7449	0.193790
Storage WeeksTreatmentLocation(Baseline)	43070	2	21535	4.4903	0.020725
Error	129490	27	4796		

Table A-27 ANOVA table - the effects of ethylene supplemented CA on peel ferulic acid in stored sweet potato (Experiment 1: 2014-15)

Effect	SS	df	MS	F	p
Intercept	237843.0	1	237843.0	213.3955	0.000000
Baseline	13487.5	1	13487.5	12.1011	0.001791
Storage Weeks(Baseline)	51993.1	1	51993.1	46.6488	0.000000
Treatment(Baseline)	15164.3	2	7582.2	6.8028	0.004205
Location(Baseline)	284.0	2	142.0	0.1274	0.880906
Storage WeeksTreatment(Baseline)	17739.9	2	8869.9	7.9582	0.002012
Storage WeeksLocation(Baseline)	3265.6	1	3265.6	2.9299	0.098856
TreatmentLocation(Baseline)	542.1	2	271.1	0.2432	0.785876
Storage WeeksTreatmentLocation(Baseline)	1386.0	2	693.0	0.6218	0.544797
Error	28978.7	26	1114.6		

Table A-28 ANOVA table - the effects of ethylene supplemented CA on flesh chlorogenic acid in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	27171308	1	27171308	1285.990	0.000000
Trans	393226	2	196613	9.305	0.000226
Storage weeks(Trans)	71312	2	35656	1.688	0.191256
Treatment(Trans)	729820	8	91228	4.318	0.000222
Location(Trans)	110118	3	36706	1.737	0.165689
Storage weeksTreatment(Trans)	279656	8	34957	1.654	0.122043
Storage weeksLocation(Trans)	2021	2	1010	0.048	0.953334
TreatmentLocation(Trans)	50489	8	6311	0.299	0.964471
Storage weeksTreatmentLocation(Trans)	114161	8	14270	0.675	0.711786
Error	1753683	83	21129		

Table A-29 ANOVA table - the effects of ethylene supplemented CA on flesh Isochlorogenic acid A in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	7829610	1	7829610	494.7363	0.000000
Trans	199165	2	99582	6.2924	0.002912
Storage weeks(Trans)	211987	2	105993	6.6975	0.002057
Treatment(Trans)	193531	8	24191	1.5286	0.160723
Location(Trans)	50173	3	16724	1.0568	0.372327
Storage weeksTreatment(Trans)	266897	8	33362	2.1081	0.044565
Storage weeksLocation(Trans)	21975	2	10988	0.6943	0.502456
TreatmentLocation(Trans)	192614	8	24077	1.5214	0.163184
Storage weeksTreatmentLocation(Trans)	149738	8	18717	1.1827	0.320090
Error	1250240	79	15826		

Table A-30 ANOVA table - the effects of ethylene supplemented CA on flesh Isochlorogenic acid B in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	256059.5	1	256059.5	165.6659	0.000000
Trans	30827.7	2	15413.9	9.9725	0.000131
Storage weeks(Trans)	731.7	2	365.9	0.2367	0.789760
Treatment(Trans)	29634.8	8	3704.3	2.3966	0.022329
Location(Trans)	3355.3	3	1118.4	0.7236	0.540724
Storage weeksTreatment(Trans)	24432.2	8	3054.0	1.9759	0.059542
Storage weeksLocation(Trans)	2060.0	2	1030.0	0.6664	0.516291
TreatmentLocation(Trans)	19754.7	8	2469.3	1.5976	0.137985
Storage weeksTreatmentLocation(Trans)	23066.8	8	2883.4	1.8655	0.076493
Error	128288.0	83	1545.6		

Table A-31 ANOVA table - the effects of ethylene supplemented CA on flesh Isochlorogenic acid C in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	2923.145	1	2923.145	31.79927	0.000000
Trans	3447.471	2	1723.735	18.75155	0.000000
Storage weeks(Trans)	69.847	2	34.924	0.37992	0.685178
Treatment(Trans)	1707.285	8	213.411	2.32158	0.027298
Location(Trans)	159.790	3	53.263	0.57942	0.630258
Storage weeksTreatment(Trans)	997.783	8	124.723	1.35679	0.228825
Storage weeksLocation(Trans)	6.779	2	3.389	0.03687	0.963816
TreatmentLocation(Trans)	1178.929	8	147.366	1.60311	0.137499
Storage weeksTreatmentLocation(Trans)	1596.407	8	199.551	2.17080	0.038726
Error	7170.145	78	91.925		

Table A-32 ANOVA table - the effects of ethylene supplemented CA on peel chlorogenic acid in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	501999195	1	501999195	1343.888	0.000000
Trans	757176	2	378588	1.014	0.367338
Storage weeks(Trans)	2615246	2	1307623	3.501	0.034654
Treatment(Trans)	45239175	8	5654897	15.139	0.000000
Location(Trans)	3737770	3	1245923	3.335	0.023233
Storage weeksTreatment(Trans)	12355532	8	1544442	4.135	0.000337
Storage weeksLocation(Trans)	838578	2	419289	1.122	0.330308
TreatmentLocation(Trans)	4808406	8	601051	1.609	0.134425
Storage weeksTreatmentLocation(Trans)	2531358	8	316420	0.847	0.564251
Error	31377558	84	373542		

Table A-33 ANOVA table - the effects of ethylene supplemented CA on peel Isochlorogenic acid A in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	1.406268E+09	1	1.406268E+09	748.8705	0.000000
Trans	4.014404E+06	2	2.007202E+06	1.0689	0.348368
Storage weeks(Trans)	5.035702E+07	2	2.517851E+07	13.4081	0.000010
Treatment(Trans)	9.444404E+07	8	1.180550E+07	6.2867	0.000003
Location(Trans)	3.806287E+07	3	1.268762E+07	6.7565	0.000413
Storage weeksTreatment(Trans)	5.145553E+07	8	6.431942E+06	3.4252	0.001998
Storage weeksLocation(Trans)	6.782640E+06	2	3.391320E+06	1.8060	0.171120
TreatmentLocation(Trans)	4.257394E+07	8	5.321742E+06	2.8340	0.008157
Storage weeksTreatmentLocation(Trans)	3.711506E+06	8	4.639382E+05	0.2471	0.980205
Error	1.464724E+08	78	1.877852E+06		

Table A-34 ANOVA table - the effects of ethylene supplemented CA on peel Isochlorogenic acid B in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	128771536	1	128771536	498.6699	0.000000
Trans	5066324	2	2533162	9.8097	0.000157
Storage weeks(Trans)	3246827	2	1623414	6.2867	0.002927
Treatment(Trans)	17103822	8	2137978	8.2794	0.000000
Location(Trans)	4919352	3	1639784	6.3501	0.000651
Storage weeksTreatment(Trans)	8181309	8	1022664	3.9603	0.000554
Storage weeksLocation(Trans)	1017961	2	508980	1.9710	0.146104
TreatmentLocation(Trans)	8759325	8	1094916	4.2401	0.000287
Storage weeksTreatmentLocation(Trans)	1677772	8	209722	0.8122	0.593964
Error	20400172	79	258230		

Table A-35 ANOVA table - the effects of peel Isochlorogenic acid C in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	17901561	1	17901561	342.3440	0.000000
Trans	2590519	2	1295260	24.7701	0.000000
Storage weeks(Trans)	507471	2	253736	4.8524	0.010323
Treatment(Trans)	1990532	8	248816	4.7583	0.000088
Location(Trans)	1209090	3	403030	7.7074	0.000142
Storage weeksTreatment(Trans)	1696047	8	212006	4.0543	0.000451
Storage weeksLocation(Trans)	30451	2	15226	0.2912	0.748196
TreatmentLocation(Trans)	1981322	8	247665	4.7363	0.000092
Storage weeksTreatmentLocation(Trans)	389356	8	48670	0.9307	0.496213
Error	4078710	78	52291		

Table A-36 ANOVA table - the effects of ethylene supplemented CA on peel caffeic acid in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	11123204	1	11123204	481.5894	0.000000
Trans	379568	2	189784	8.2169	0.000551
Storage weeks(Trans)	122688	2	61344	2.6559	0.076127
Treatment(Trans)	1730010	8	216251	9.3628	0.000000
Location(Trans)	48034	3	16011	0.6932	0.558720
Storage weeksTreatment(Trans)	420283	8	52535	2.2746	0.029656
Storage weeksLocation(Trans)	638	2	319	0.0138	0.986279
TreatmentLocation(Trans)	98916	8	12365	0.5353	0.826752
Storage weeksTreatmentLocation(Trans)	33230	8	4154	0.1798	0.993084
Error	1940137	84	23097		

Table A-37 ANOVA table - the effects of ethylene supplemented CA on peel ferulic acid in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	75121.88	1	75121.88	149.7601	0.000000
Trans	29912.25	2	14956.12	29.8159	0.000000
Storage weeks(Trans)	6075.40	2	3037.70	6.0558	0.003556
Treatment(Trans)	4585.05	8	573.13	1.1426	0.344544
Location(Trans)	9028.57	3	3009.52	5.9997	0.000969
Storage weeksTreatment(Trans)	21650.58	8	2706.32	5.3952	0.000019
Storage weeksLocation(Trans)	17.27	2	8.63	0.0172	0.982938
TreatmentLocation(Trans)	10250.28	8	1281.29	2.5543	0.015621
Storage weeksTreatmentLocation(Trans)	4899.45	8	612.43	1.2209	0.297706
Error	40129.19	80	501.61		

Table A-38 ANOVA table – the effects of ethylene supplemented CA on flesh ABA concentration in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	10955843	1	10955843	253.8506	0.000000
Baseline	2423692	1	2423692	56.1578	0.000000
Outturn (weeks)(Baseline)	453772	1	453772	10.5140	0.003145
Treatment(Baseline)	737857	2	368929	8.5482	0.001330
Location(Baseline)	24476	2	12238	0.2836	0.755313
Outturn (weeks)Treatment(Baseline)	29855	2	14927	0.3459	0.710692
Outturn (weeks)Location(Baseline)	33364	1	33364	0.7731	0.387033
TreatmentLocation(Baseline)	2965	2	1482	0.0343	0.966280
Outturn (weeks)TreatmentLocation(Baseline)	32938	2	16469	0.3816	0.686395
Error	1165283	27	43159		

Table A-39 ANOVA table - the effects of ethylene supplemented CA on peel ABA concentration in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	10764343	1	10764343	178.9256	0.000000
Baseline	1978959	1	1978959	32.8944	0.000004
Outturn (weeks)(Baseline)	23096	1	23096	0.3839	0.540715
Treatment(Baseline)	514354	2	257177	4.2748	0.024386
Location(Baseline)	137787	2	68893	1.1451	0.333150
Outturn (weeks)Treatment(Baseline)	120761	2	60380	1.0036	0.379807
Outturn (weeks)Location(Baseline)	50805	1	50805	0.8445	0.366255
TreatmentLocation(Baseline)	1463	2	731	0.0122	0.987921
Outturn (weeks)TreatmentLocation(Baseline)	7532	2	3766	0.0626	0.939460
Error	1624347	27	60161		

Table A-40 ANOVA table - the effects of ethylene supplemented CA on flesh zeatin riboside in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	56825.51	1	56825.51	39.06589	0.000001
Baseline	8882.31	1	8882.31	6.10633	0.020071
Outturn (weeks)(Baseline)	7435.86	1	7435.86	5.11194	0.032029
Treatment(Baseline)	5040.44	2	2520.22	1.73258	0.195913
Location(Baseline)	2131.25	2	1065.62	0.73259	0.489976
Outturn (weeks)Treatment(Baseline)	7145.07	2	3572.54	2.45602	0.104719
Outturn (weeks)Location(Baseline)	5.02	1	5.02	0.00345	0.953572
TreatmentLocation(Baseline)	1613.53	2	806.77	0.55463	0.580690
Outturn (weeks)TreatmentLocation(Baseline)	16512.60	2	8256.30	5.67597	0.008756
Error	39274.38	27	1454.61		

Table A-41 ANOVA table - the effects of ethylene supplemented CA on peel zeatin riboside in stored sweet potato (Experiment 2: 2015-16)

Effect	SS	df	MS	F	p
Intercept	114632.5	1	114632.5	19.39836	0.000151
Baseline	6223.9	1	6223.9	1.05322	0.313875
Outturn (weeks)(Baseline)	1037.1	1	1037.1	0.17550	0.678587
Treatment(Baseline)	38784.6	2	19392.3	3.28161	0.052997
Location(Baseline)	137.8	2	68.9	0.01166	0.988417
Outturn (weeks)Treatment(Baseline)	13016.5	2	6508.2	1.10134	0.346900
Outturn (weeks)Location(Baseline)	1931.4	1	1931.4	0.32684	0.572254
TreatmentLocation(Baseline)	1816.7	2	908.4	0.15372	0.858260
Outturn (weeks)TreatmentLocation(Baseline)	6221.7	2	3110.8	0.52642	0.596655
Error	159553.5	27	5909.4		

Appendix B – Chapter four: Fungal growth and aflatoxin development on sweet potato stored under controlled atmosphere conditions

Table B-1 ANOVA table - the effects of CA conditions on disease incidence (%) in stored sweet potato

Effect	SS	df	MS	F	p
Intercept	424.4770	1	424.4770	171.3842	0.000000
Baseline	257.2016	1	257.2016	103.8462	0.000000
Treatment(Baseline)	115.0739	2	57.5370	23.2308	0.000002
Location(Baseline)	290.3521	4	72.5880	29.3077	0.000000
TreatmentLocation(Baseline)	49.5351	4	12.3838	5.0000	0.004477
Error	59.4422	24	2.4768		

Table B-2 ANOVA table - the effects of CA storage conditions on fungal counts in DG-18 culture media (cfu/mg) during sweet potato storage

Effect	SS	df	MS	F	p
Intercept	3696.001	1	3696.001	458.0786	0.000000
Baseline	134.320	1	134.320	16.6475	0.000230
Time (Wks)(Baseline)	1173.722	1	1173.722	145.4699	0.000000
Treatment(Baseline)	4255.176	2	2127.588	263.6911	0.000000
Location(Baseline)	755.981	4	188.995	23.4239	0.000000
Time (Wks)Treatment(Baseline)	2420.242	2	1210.121	149.9811	0.000000
Time (Wks)Location(Baseline)	514.531	2	257.265	31.8852	0.000000
TreatmentLocation(Baseline)	2075.416	4	518.854	64.3062	0.000000
Time (Wks)TreatmentLocation(Baseline)	1206.663	4	301.666	37.3881	0.000000
Error	298.534	37	8.068		

Table B-3 ANOVA table - the effects of CA storage conditions on fungal counts (cfu/mg) in PDA culture media during sweet potato storage

Effect	SS	df	MS	F	p
Intercept	3680.958	1	3680.958	231.8875	0.000000
Baseline	133.841	1	133.841	8.4315	0.006264
Time (Wks)(Baseline)	1190.440	1	1190.440	74.9936	0.000000
Treatment(Baseline)	2535.144	2	1267.572	79.8526	0.000000
Location(Baseline)	397.362	4	99.340	6.2581	0.000626
Time (Wks)Treatment(Baseline)	1146.595	2	573.298	36.1157	0.000000
Time (Wks)Location(Baseline)	87.322	2	43.661	2.7505	0.077339
TreatmentLocation(Baseline)	767.167	4	191.792	12.0822	0.000003
Time (Wks)TreatmentLocation(Baseline)	133.971	4	33.493	2.1099	0.099732
Error	571.460	36	15.874		

Table B-4 ANOVA table - the effects of CA storage on *Fusarium* development on stored sweet potato cultured in DG-18 media

Effect	SS	df	MS	F	p
Intercept	51552.97	1	51552.97	89.02670	0.000000
Baseline	15742.87	1	15742.87	27.18633	0.000024
Treatment(Baseline)	1061.68	2	530.84	0.91671	0.413384
Location(Baseline)	3295.25	4	823.81	1.42264	0.256815
TreatmentLocation(Baseline)	11618.86	4	2904.71	5.01614	0.004405
Error	13897.75	24	579.07		

Table B-5 ANOVA table - the effects of CA storage on *Penicilium* development in stored sweet potato cultured in DG-18 media

Effect	SS	df	MS	F	p
Intercept	83173.84	1	83173.84	148.3405	0.000000
Baseline	25545.27	1	25545.27	45.5600	0.000001
Treatment(Baseline)	1501.53	2	750.77	1.3390	0.280995
Location(Baseline)	2618.78	4	654.69	1.1676	0.349811
TreatmentLocation(Baseline)	4664.89	4	1166.22	2.0800	0.114956
Error	13456.69	24	560.70		

Table B-6 ANOVA table - the effects of CA storage on *Aspergillus* development in stored sweet potato cultured in DG-18 media

Effect	SS	df	MS	F	p
Intercept	3.85906	1	3.859062	1.150578	0.294096
Baseline	0.10686	1	0.106862	0.031861	0.859832
Treatment(Baseline)	8.50340	2	4.251701	1.267643	0.299674
Location(Baseline)	9.82537	4	2.456341	0.732357	0.578831
TreatmentLocation(Baseline)	17.00680	4	4.251701	1.267643	0.310086
Error	80.49651	24	3.354021		

Table B-7 ANOVA table - the effects of CA storage *Rhizopus* devlopment in stored sweet potato cultured in DG-18 media

Effect	SS	df	MS	F	p
Intercept	165.885	1	165.8848	3.102253	0.090921
Baseline	36.465	1	36.4649	0.681940	0.417053
Treatment(Baseline)	157.505	2	78.7524	1.472768	0.249283
Location(Baseline)	171.355	4	42.8388	0.801139	0.536401
TreatmentLocation(Baseline)	710.969	4	177.7423	3.324003	0.026611
Error	1283.337	24	53.4724		

Table B-8 ANOVA table - the effects of CA storage on *Fusarium* development in stored sweet potato cultured in PDA

Effect	SS	df	MS	F	p
Intercept	155865.9	1	155865.9	282.5251	0.000000
Baseline	2445.2	1	2445.2	4.4323	0.045916
Treatment(Baseline)	4542.8	2	2271.4	4.1171	0.029021
Location(Baseline)	2818.2	4	704.5	1.2771	0.306567
TreatmentLocation(Baseline)	3319.9	4	830.0	1.5044	0.232387
Error	13240.5	24	551.7		

Table B-9 the effects of CA storage on *Penicilium* development in sweet potato cultured in PDA

Effect	SS	df	MS	F	p
Intercept	25754.03	1	25754.03	50.23067	0.000000
Baseline	2436.09	1	2436.09	4.75136	0.039316
Treatment(Baseline)	4418.76	2	2209.38	4.30917	0.025176
Location(Baseline)	3870.39	4	967.60	1.88721	0.145410
TreatmentLocation(Baseline)	3142.48	4	785.62	1.53227	0.224597
Error	12305.16	24	512.72		

Table B-10 ANOVA table - the effects of CA storage on *Aspergillus* development in sweet potato cultured in PDA

Effect	SS	df	MS	F	p
Intercept	0.51830	1	0.518303	0.571429	0.457048
Baseline	0.30234	1	0.302343	0.333333	0.569080
Treatment(Baseline)	2.41875	2	1.209373	1.333333	0.282430
Location(Baseline)	2.41875	4	0.604686	0.666667	0.621345
TreatmentLocation(Baseline)	4.83749	4	1.209373	1.333333	0.286334
Error	21.76871	24	0.907029		

Table B-11 ANOVA table - the effects of CA storage on *Rhizopus* development in sweet potato cultured in PDA

Effect	SS	df	MS	F	p
Intercept	119.8513	1	119.8513	7.933299	0.009553
Baseline	0.4128	1	0.4128	0.027325	0.870091
Treatment(Baseline)	181.2494	2	90.6247	5.998704	0.007714
Location(Baseline)	173.2113	4	43.3028	2.866336	0.045004
TreatmentLocation(Baseline)	190.7672	4	47.6918	3.156855	0.032179
Error	362.5770	24	15.1074		

Table B-12 ANOVA table - the effects of CA storage on aflatoxin B1 production in sweet potato

Effect	SS	df	MS	F	p
Intercept	240.2854	1	240.2854	170.8523	0.000012
Baseline	40.7827	1	40.7827	28.9981	0.001688
Outturn(Baseline)	15.6898	1	15.6898	11.1560	0.015610
Treatment(Baseline)	44.5873	1	44.5873	31.7033	0.001343
Location(Baseline)	124.4437	1	124.4437	88.4843	0.000082
OutturnTreatmentLocation(Baseline)	9.5820	1	9.5820	6.8132	0.040109
Error	8.4384	6	1.4064		

Table B-13 ANOVA table - the effects of CA storage on aflatoxin B2 production in sweet potato

Effect	SS	df	MS	F	p
Intercept	504.2827	1	504.2827	324.1746	0.000002
Baseline	295.2574	1	295.2574	189.8042	0.000009
Outturn(Baseline)	80.1209	1	80.1209	51.5052	0.000370
Treatment(Baseline)	103.0126	1	103.0126	66.2209	0.000185
Location(Baseline)	11.4458	1	11.4458	7.3579	0.034988
OutturnTreatmentLocation(Baseline)	80.1209	1	80.1209	51.5052	0.000370
Error	9.3335	6	1.5556		

Table B-14 ANOVA table - the effects of CA storage on aflatoxin G1 production in sweet potato

Effect	SS	df	MS	F	p
Intercept	9816.593	1	9816.593	1437.255	0.000000
Baseline	1811.820	1	1811.820	265.270	0.000003
Outturn(Baseline)	684.442	1	684.442	100.210	0.000058
Treatment(Baseline)	1407.394	1	1407.394	206.058	0.000007
Location(Baseline)	7654.896	1	7654.896	1120.759	0.000000
OutturnTreatmentLocation(Baseline)	4446.162	1	4446.162	650.966	0.000000
Error	40.981	6	6.830		

Appendix C – Chapter Five: Molecular regulation of metabolic biosynthesis in sweet potato stored under controlled atmosphere and ethylene conditions

Table C-1 ANOVA table - the effects of CA and ethylene storage on flesh fructose accumulation in sweet potato

Effect	SS	df	MS	F	p
Intercept	341998.4	1	341998.4	3437.089	0.000000
Transition	2385.2	2	1192.6	11.986	0.000039
Time(Transition)	784.9	2	392.5	3.944	0.024326
Treatments(Transition)	5544.4	8	693.0	6.965	0.000002
Location(Transition)	591.4	3	197.1	1.981	0.125824
TimeTreatments(Transition)	6201.6	4	1550.4	15.581	0.000000
TimeLocation(Transition)	2.9	2	1.5	0.015	0.985454
TreatmentsLocation(Transition)	472.2	8	59.0	0.593	0.779855
TimeTreatmentsLocation(Transition)	333.0	4	83.2	0.837	0.507108
Error	6268.6	63	99.5		

Table C-2 ANOVA table - the effects of CA and ethylene on flesh glucose accumulation during storage of sweet potato

Effect	SS	df	MS	F	p
Intercept	374364.7	1	374364.7	4501.482	0.000000
Transition	7280.2	2	3640.1	43.770	0.000000
Time(Transition)	5041.9	2	2521.0	30.313	0.000000
Treatments(Transition)	6663.7	8	833.0	10.016	0.000000
Location(Transition)	219.5	3	73.2	0.880	0.456437
TimeTreatments(Transition)	3195.0	4	798.8	9.604	0.000004
TimeLocation(Transition)	7.6	2	3.8	0.046	0.955494
TreatmentsLocation(Transition)	472.8	8	59.1	0.711	0.681180
TimeTreatmentsLocation(Transition)	217.9	4	54.5	0.655	0.625440
Error	5239.4	63	83.2		

Table C-3 ANOVA table - the effects of CA and ethylene on flesh sucrose accumulation during storage of sweet potato

Effect	SS	df	MS	F	p
Intercept	190105.1	1	190105.1	2567.757	0.000000
Transition	15409.2	2	7704.6	104.066	0.000000
Time(Transition)	17803.4	2	8901.7	120.236	0.000000
Treatments(Transition)	11465.8	8	1433.2	19.359	0.000000
Location(Transition)	222.5	3	74.2	1.002	0.397931
TimeTreatments(Transition)	3217.0	4	804.3	10.863	0.000001
TimeLocation(Transition)	110.5	2	55.3	0.746	0.478149
TreatmentsLocation(Transition)	851.9	8	106.5	1.438	0.198054
TimeTreatmentsLocation(Transition)	570.9	4	142.7	1.928	0.116518
Error	4738.3	64	74.0		

Table C-4 ANOVA table - the effects of CA and ethylene on peel fructose accumulation during storage of sweet potato

Effect	SS	df	MS	F	p
Intercept	8581.270	1	8581.270	895.5262	0.000000
Transition	4765.999	2	2382.999	248.6856	0.000000
Time(Transition)	1848.339	2	924.170	96.4447	0.000000
Treatments(Transition)	332.695	8	41.587	4.3399	0.000304
Location(Transition)	121.115	3	40.372	4.2131	0.008683
TimeTreatments(Transition)	648.949	4	162.237	16.9308	0.000000
TimeLocation(Transition)	2.187	2	1.093	0.1141	0.892338
TreatmentsLocation(Transition)	138.173	8	17.272	1.8024	0.092292
TimeTreatmentsLocation(Transition)	11.655	4	2.914	0.3041	0.874251
Error	632.437	66	9.582		

Table C-5 ANOVA table - the effects of CA and ethylene on peel glucose accumulation during storage of sweet potato

Effect	SS	df	MS	F	p
Intercept	11527.97	1	11527.97	1877.372	0.000000
Transition	1173.52	2	586.76	95.556	0.000000
Time(Transition)	757.41	2	378.71	61.674	0.000000
Treatments(Transition)	491.01	8	61.38	9.995	0.000000
Location(Transition)	19.26	3	6.42	1.046	0.378456
TimeTreatments(Transition)	235.03	4	58.76	9.569	0.000004
TimeLocation(Transition)	38.99	2	19.50	3.175	0.048342
TreatmentsLocation(Transition)	202.47	8	25.31	4.122	0.000505
TimeTreatmentsLocation(Transition)	93.18	4	23.29	3.793	0.007816
Error	399.13	65	6.14		

Table C-6 ANOVA table - the effects of CA and ethylene on peel sucrose accumulation during storage of sweet potato

Effect	SS	df	MS	F	p
Intercept	98781.29	1	98781.29	1457.501	0.000000
Transition	1095.76	2	547.88	8.084	0.000716
Time(Transition)	1984.02	2	992.01	14.637	0.000005
Treatments(Transition)	24590.15	8	3073.77	45.353	0.000000
Location(Transition)	426.83	3	142.28	2.099	0.108564
TimeTreatments(Transition)	4687.39	4	1171.85	17.290	0.000000
TimeLocation(Transition)	18.77	2	9.39	0.138	0.870915
TreatmentsLocation(Transition)	116.11	8	14.51	0.214	0.987355
TimeTreatmentsLocation(Transition)	290.26	4	72.56	1.071	0.378005
Error	4540.89	67	67.77		

Table C-7 ANOVA table - the effects of CA and ethylene on flesh chlorogenic acid during sweet storage

Effect	SS	df	MS	F	p
Intercept	38257793	1	38257793	845.3510	0.000000
Transition	526416	2	263208	5.8159	0.004688
Time(Transition)	155933	2	77966	1.7228	0.186388
Treatment(Transition)	898080	8	112260	2.4805	0.020248
Location(Transition)	159996	3	53332	1.1784	0.324592
TimeTreatment(Transition)	846578	4	211645	4.6765	0.002174
TimeLocation(Transition)	66092	2	33046	0.7302	0.485613
TreatmentLocation(Transition)	764526	8	95566	2.1116	0.046585
TimeTreatmentLocation(Transition)	33149	4	8287	0.1831	0.946399
Error	3032199	67	45257		

Table C-8 ANOVA table - the effects of CA and ethylene on flesh Isochlorogenic acid A during sweet potato storage

Effect	SS	df	MS	F	p
Intercept	1313113	1	1313113	308.7467	0.000000
Transition	117541	2	58770	13.8184	0.000011
Time(Transition)	130461	2	65230	15.3373	0.000004
Treatment(Transition)	264685	8	33086	7.7793	0.000000
Location(Transition)	27839	3	9280	2.1819	0.099094
TimeTreatment(Transition)	102948	4	25737	6.0515	0.000354
TimeLocation(Transition)	15328	2	7664	1.8020	0.173494
TreatmentLocation(Transition)	69814	8	8727	2.0519	0.054561
TimeTreatmentLocation(Transition)	6587	4	1647	0.3872	0.816994
Error	263689	62	4253		

Table C-9 ANOVA table - the effects of CA and ethylene on flesh Isochlorogenic acid B during sweet potato storage

Effect	SS	df	MS	F	p
Intercept	20965101	1	20965101	1083.528	0.000000
Transition	430382	2	215191	11.122	0.000070
Time(Transition)	196456	2	98228	5.077	0.008941
Treatment(Transition)	485003	8	60625	3.133	0.004663
Location(Transition)	90331	3	30110	1.556	0.208510
TimeTreatment(Transition)	199823	4	49956	2.582	0.045245
TimeLocation(Transition)	20557	2	10279	0.531	0.590413
TreatmentLocation(Transition)	93995	8	11749	0.607	0.768503
TimeTreatmentLocation(Transition)	53821	4	13455	0.695	0.597856
Error	1257680	65	19349		

Table C-10 ANOVA table - the effects of CA and theylene on flesh Isochlorogenic acid C during sweet potato storage

Effect	SS	df	MS	F	p
Intercept	8841.251	1	8841.251	79.12900	0.000000
Transition	250.443	2	125.222	1.12073	0.332355
Time(Transition)	437.041	2	218.521	1.95575	0.149820
Treatment(Transition)	1132.225	8	141.528	1.26667	0.276655
Location(Transition)	241.988	3	80.663	0.72193	0.542576
TimeTreatment(Transition)	224.910	4	56.227	0.50323	0.733445
TimeLocation(Transition)	61.299	2	30.649	0.27431	0.760985
TreatmentLocation(Transition)	1166.528	8	145.816	1.30505	0.257157
TimeTreatmentLocation(Transition)	384.193	4	96.048	0.85963	0.493085
Error	7150.855	64	111.732		

Table C-11 ANOVA table - the effects of CA and ethylene on peel chlorogenic acid during sweet potato storage

Effect	SS	df	MS	F	p
Intercept	7.321903E+09	1	7.321903E+09	5459.057	0.000000
Transition	9.184106E+07	2	4.592053E+07	34.237	0.000000
Time(Transition)	3.130144E+07	2	1.565072E+07	11.669	0.000044
Treatment(Transition)	1.231997E+08	8	1.539996E+07	11.482	0.000000
Location(Transition)	2.285797E+06	3	7.619325E+05	0.568	0.637932
TimeTreatment(Transition)	5.157907E+07	4	1.289477E+07	9.614	0.000003
TimeLocation(Transition)	2.818403E+06	2	1.409202E+06	1.051	0.355310
TreatmentLocation(Transition)	1.217261E+07	8	1.521577E+06	1.134	0.351960
TimeTreatmentLocation(Transition)	1.632923E+06	4	4.082308E+05	0.304	0.874083
Error	9.120429E+07	68	1.341240E+06		

Table C-12 ANOVA table - the effects of CA and ethylene on peel Isochlorogenic acid A during sweet potato storage

Effect	SS	df	MS	F	p
Intercept	2.117304E+09	1	2.117304E+09	1377.232	0.000000
Transition	1.398341E+08	2	6.991703E+07	45.479	0.000000
Time(Transition)	1.565181E+08	2	7.825907E+07	50.905	0.000000
Treatment(Transition)	5.034490E+07	8	6.293112E+06	4.093	0.000513
Location(Transition)	1.941113E+06	3	6.470376E+05	0.421	0.738620
TimeTreatment(Transition)	1.305940E+06	4	3.264850E+05	0.212	0.930700
TimeLocation(Transition)	1.067967E+06	2	5.339834E+05	0.347	0.707832
TreatmentLocation(Transition)	8.485890E+06	8	1.060736E+06	0.690	0.698908
TimeTreatmentLocation(Transition)	4.502850E+06	4	1.125712E+06	0.732	0.573159
Error	1.030033E+08	67	1.537362E+06		

Table C-13 ANOVA table - the effects of CA and ethylene on peel Isochlorogenic acid B during storage of sweet potato

Effect	SS	df	MS	F	p
Intercept	1.321751E+10	1	1.321751E+10	599.4346	0.000000
Transition	3.813300E+08	2	1.906650E+08	8.6470	0.000451
Time(Transition)	1.865960E+09	2	9.329800E+08	42.3121	0.000000
Treatment(Transition)	2.303034E+09	8	2.878792E+08	13.0558	0.000000
Location(Transition)	1.342922E+07	3	4.476405E+06	0.2030	0.893967
TimeTreatment(Transition)	1.397017E+09	4	3.492543E+08	15.8392	0.000000
TimeLocation(Transition)	1.169059E+06	2	5.845295E+05	0.0265	0.973849
TreatmentLocation(Transition)	5.372587E+08	8	6.715734E+07	3.0457	0.005484
TimeTreatmentLocation(Transition)	4.747746E+07	4	1.186936E+07	0.5383	0.708072
Error	1.499398E+09	68	2.204996E+07		

Table C-14 ANOVA table - the effects of CA and ethylene on Isochlorogenic acid C during storage of sweet potato

Effect	SS	df	MS	F	p
Intercept	603310284	1	603310284	1250.860	0.000000
Transition	138469514	2	69234757	143.546	0.000000
Time(Transition)	161690589	2	80845294	167.619	0.000000
Treatment(Transition)	69385819	8	8673227	17.982	0.000000
Location(Transition)	208134	3	69378	0.144	0.933262
TimeTreatment(Transition)	6022561	4	1505640	3.122	0.020644
TimeLocation(Transition)	1272455	2	636227	1.319	0.274435
TreatmentLocation(Transition)	3052323	8	381540	0.791	0.612307
TimeTreatmentLocation(Transition)	829599	4	207400	0.430	0.786439
Error	31350560	65	482316		

Table C-15 ANOVA table - the effects of CA and ethylene on peel caffeic acid during sweet potato storage

Effect	SS	df	MS	F	p
Intercept	161270903	1	161270903	1710.060	0.000000
Transition	3090928	2	1545464	16.388	0.000002
Time(Transition)	1182908	2	591454	6.272	0.003165
Treatment(Transition)	593582	8	74198	0.787	0.615882
Location(Transition)	182976	3	60992	0.647	0.587696
TimeTreatment(Transition)	300966	4	75242	0.798	0.530753
TimeLocation(Transition)	57396	2	28698	0.304	0.738636
TreatmentLocation(Transition)	461976	8	57747	0.612	0.764473
TimeTreatmentLocation(Transition)	88459	4	22115	0.234	0.917995
Error	6412885	68	94307		

Table C-16 ANOVA table - the effects of CA and ethylene on peel ferulic acid during storage of sweet potato

Effect	SS	df	MS	F	p
Intercept	1210157	1	1210157	2334.992	0.000000
Transition	1908	2	954	1.841	0.166512
Time(Transition)	5303	2	2651	5.116	0.008519
Treatment(Transition)	4359	8	545	1.051	0.407385
Location(Transition)	1088	3	363	0.700	0.555400
TimeTreatment(Transition)	3298	4	825	1.591	0.186715
TimeLocation(Transition)	796	2	398	0.768	0.467853
TreatmentLocation(Transition)	2757	8	345	0.665	0.720216
TimeTreatmentLocation(Transition)	2143	4	536	1.034	0.396343
Error	35242	68	518		

Table C-17 ANOVA table - the effects of CA and ethylene on flesh ABA (sq rt) concentration in sweet potato during storage

Effect	SS	df	MS	F	p
Intercept	113693.1	1	113693.1	3808.789	0.000000
Baseline	6.1	1	6.1	0.203	0.654127
Time(Baseline)	16134.1	4	4033.5	135.125	0.000000
Treatment(Baseline)	33536.5	4	8384.1	280.873	0.000000
Location(Baseline)	45.4	2	22.7	0.760	0.473491
TimeTreatment(Baseline)	12884.4	7	1840.6	61.662	0.000000
TimeLocation(Baseline)	56.2	4	14.1	0.471	0.756734
TreatmentLocation(Baseline)	89.0	4	22.3	0.745	0.566068
TimeTreatmentLocation(Baseline)	146.3	7	20.9	0.700	0.671476
Error	1373.1	46	29.9		

Table C-18 ANOVA table - the effects of CA and ethylene on peel ABA (sq rt) concentration during storage of sweet potato

Effect	SS	df	MS	F	p
Intercept	60109.49	1	60109.49	292.8506	0.000000
Baseline	1458.07	1	1458.07	7.1037	0.010448
Time(Baseline)	2684.89	4	671.22	3.2702	0.018891
Treatment(Baseline)	4505.76	4	1126.44	5.4880	0.001013
Location(Baseline)	557.32	2	278.66	1.3576	0.266971
TimeTreatment(Baseline)	1601.56	7	228.79	1.1147	0.369553
TimeLocation(Baseline)	2046.52	4	511.63	2.4926	0.055303
TreatmentLocation(Baseline)	5049.28	4	1262.32	6.1500	0.000445
TimeTreatmentLocation(Baseline)	3113.40	7	444.77	2.1669	0.054073
Error	9852.31	48	205.26		

Table C-19 ANOVA table - the effects of CA and ethylene on flesh PA (ng/g) during storage of sweet potato

Effect	SS	df	MS	F	p
Intercept	443294.9	1	443294.9	443.5179	0.000000
Baseline	12045.3	1	12045.3	12.0513	0.001090
Time(Baseline)	53052.4	3	17684.1	17.6930	0.000000
Treatment(Baseline)	60667.8	4	15167.0	15.1746	0.000000
Location(Baseline)	9991.3	2	4995.7	4.9982	0.010583
TimeTreatment(Baseline)	43193.6	5	8638.7	8.6431	0.000006
TimeLocation(Baseline)	2200.3	3	733.4	0.7338	0.536871
TreatmentLocation(Baseline)	10704.2	4	2676.1	2.6774	0.042527
TimeTreatmentLocation(Baseline)	16458.7	5	3291.7	3.2934	0.012137
Error	48975.4	49	999.5		

Table C-20 ANOVA table - the effects of CA and ethylene on peel PA (ng/g) during storage of sweet potato

Effect	SS	df	MS	F	p
Intercept	4599946	1	4599946	325.1410	0.000000
Baseline	2957	1	2957	0.2090	0.649354
Time(Baseline)	475184	3	158395	11.1959	0.000008
Treatment(Baseline)	368127	4	92032	6.5051	0.000239
Location(Baseline)	53528	2	26764	1.8918	0.160664
TimeTreatment(Baseline)	245540	5	49108	3.4711	0.008591
TimeLocation(Baseline)	8720	3	2907	0.2054	0.892192
TreatmentLocation(Baseline)	68166	4	17042	1.2046	0.319560
TimeTreatmentLocation(Baseline)	188918	5	37784	2.6707	0.031465
Error	763967	54	14148		

Table C-21 ANOVA table - the effects of CA and ethylene on flesh trans-zeatin riboside (Log) during storage of sweet potato

Effect	SS	df	MS	F	p
Intercept	70.01501	1	70.01501	76.13356	0.000000
Baseline	7.68890	1	7.68890	8.36082	0.008469
Time(Baseline)	2.29867	3	0.76622	0.83318	0.489959
Treatment(Baseline)	10.18789	4	2.54697	2.76955	0.052760
Location(Baseline)	8.96872	2	4.48436	4.87625	0.017664
TimeTreatment(Baseline)	0.32786	2	0.16393	0.17826	0.837925
TimeLocation(Baseline)	0.24297	2	0.12149	0.13210	0.876940
TreatmentLocation(Baseline)	0.35382	3	0.11794	0.12825	0.942319
Error	20.23195	22	0.91963		

Table C-22 ANOVA table - the effects of CA and ethylene on trans-zeatin riboside (Log) during storage of sweet potato

Effect	SS	df	MS	F	p
Intercept	193.9305	1	193.9305	683.3811	0.000000
Baseline	5.2431	1	5.2431	18.4759	0.000100
Time(Baseline)	14.9372	3	4.9791	17.5455	0.000000
Treatment(Baseline)	1.1002	4	0.2750	0.9692	0.434475
Location(Baseline)	1.4389	2	0.7195	2.5353	0.091303
TimeTreatment(Baseline)	4.4352	5	0.8870	3.1258	0.017330
TimeLocation(Baseline)	1.2887	3	0.4296	1.5138	0.224886
TreatmentLocation(Baseline)	0.7891	4	0.1973	0.6952	0.599522
TimeTreatmentLocation(Baseline)	4.0320	5	0.8064	2.8416	0.026792
Error	11.9188	42	0.2838		

Table C-23 - ANOVA table - the effects of CA and ethylene on the relative expression of ERF gene in the flesh of sweet potato during storage

Effect	SS	df	MS	F	p
Intercept	23.02013	1	23.02013	50.56051	0.000000
Baseline	1.03562	1	1.03562	2.27459	0.137449
Time(Baseline)	38.96672	3	12.98891	28.52832	0.000000
Treatment(Baseline)	17.76208	4	4.44052	9.75298	0.000005
Location(Baseline)	1.98901	2	0.99450	2.18429	0.122588
TimeTreatment(Baseline)	15.05474	5	3.01095	6.61312	0.000075
TimeLocation(Baseline)	10.39736	3	3.46579	7.61211	0.000253
TreatmentLocation(Baseline)	5.88669	4	1.47167	3.23232	0.019050
TimeTreatmentLocation(Baseline)	10.11959	5	2.02392	4.44525	0.001869
Error	24.13083	53	0.45530		

Table C-24 ANOVA table - the effects of CA and ethylene on the relative expression of EIN2 in the flesh of sweet potato during storage

Effect	SS	df	MS	F	p
Intercept	13.04293	1	13.04293	1592.208	0.000000
Baseline	2.10038	1	2.10038	256.403	0.000000
Time(Baseline)	13.98339	3	4.66113	569.005	0.000000
Treatment(Baseline)	0.65723	4	0.16431	20.058	0.000000
Location(Baseline)	1.56350	2	0.78175	95.432	0.000000
TimeTreatment(Baseline)	1.16190	5	0.23238	28.368	0.000000
TimeLocation(Baseline)	3.81381	3	1.27127	155.190	0.000000
TreatmentLocation(Baseline)	0.24835	4	0.06209	7.579	0.000177
TimeTreatmentLocation(Baseline)	0.37532	5	0.07506	9.163	0.000014
Error	0.27852	34	0.00819		

Table C-25 ANOVA table - the effects of CA and ethylene on the relative expression of ERF gene in the peel of sweet potato during storage

Effect	SS	df	MS	F	p
Intercept	65.22023	1	65.22023	105.4461	0.000000
Baseline	0.31684	1	0.31684	0.5123	0.477629
Time(Baseline)	5.52043	3	1.84014	2.9751	0.040764
Treatment(Baseline)	7.20417	4	1.80104	2.9119	0.030933
Location(Baseline)	5.54209	2	2.77104	4.4801	0.016446
TimeTreatment(Baseline)	49.29456	5	9.85891	15.9396	0.000000
TimeLocation(Baseline)	29.01645	3	9.67215	15.6376	0.000000
TreatmentLocation(Baseline)	33.41292	4	8.35323	13.5053	0.000000
TimeTreatmentLocation(Baseline)	14.92346	5	2.98469	4.8256	0.001181
Error	29.68881	48	0.61852		

Table C-26 ANOVA table - the effects of CA and ethylene on the relative expression of EIN2 gene in the peel of sweet potato during storage

Effect	SS	df	MS	F	p
Intercept	34.19905	1	34.19905	153.0382	0.000000
Baseline	0.24518	1	0.24518	1.0971	0.300250
Time(Baseline)	73.81108	3	24.60369	110.0997	0.000000
Treatment(Baseline)	0.71631	4	0.17908	0.8014	0.530478
Location(Baseline)	7.65532	2	3.82766	17.1285	0.000003
TimeTreatment(Baseline)	1.17340	5	0.23468	1.0502	0.399833
TimeLocation(Baseline)	16.10533	3	5.36844	24.0234	0.000000
TreatmentLocation(Baseline)	2.74460	4	0.68615	3.0705	0.025071
TimeTreatmentLocation(Baseline)	6.41931	5	1.28386	5.7452	0.000324
Error	10.50297	47	0.22347		

Appendix D – Agar plates – Isolated fungal spores

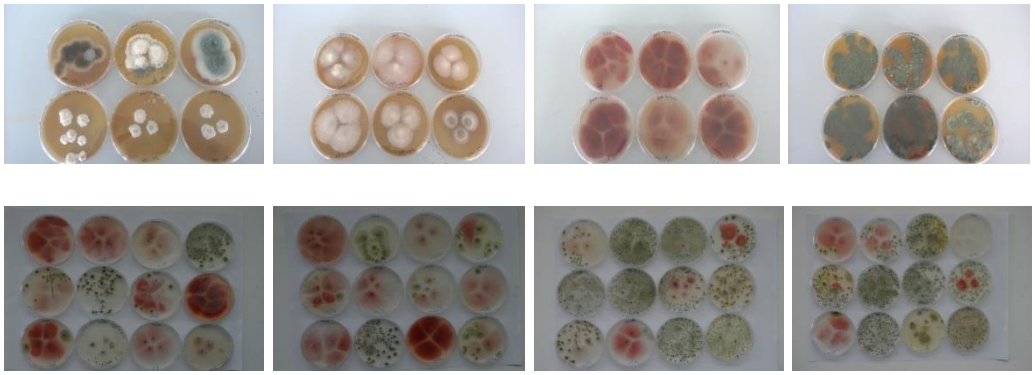


Figure D1 - Agar plates for identification of fungal species in sweet potato during CA storage

Appendix E – Quantitative polymerase chain reaction (qPCR) analyses

Table E-1 List of primers tested for the quantitative polymerase chain reaction

Name of the gene	Forward Primer	Reverse Primer	Product size(bp)
Ethylene insensitive2 (EIN2)	CGAAGGTTCTGACTGGCTGT	TCTGGCCTGCTTTCCATGAG	318
	GGATGATGGAGCTGACGAGG	ATTGGCGAATGAGGCTTGGA	372
	TCATGGAAAGCAGGCCAGAG	GCATTTTCCGCGGTTCTGTT	212
	TCGAGTACTAGGGCACCTCC	GGGCGATAAGCCATCTGTGA	293
1-aminocyclopropane-1-carboxylate oxidase (ACO)	TCTCCAGCTTCTGAAAGACGG	GGTAAACTTTCTCATCATCTCCCAC	248
	CGAGGTAATCGCCAACGGTAA	TTGGGTAAACTTTCTCATCATCTCC	170
	GAAGGTGTACAAGCCGAGGT	CTAGCCCCGTTTGTTTGTGC	509
	CATGCCCAAAACCCGACTTG	CTAGCCCCGTTTGTTTGTGC	241
1-aminocyclopropane-1-carboxylate synthase (ACS)	TCCGCAGCCACAAAAATGTC	GGCATGACGATCCAGGTGAA	316
	CCCTTCGAACCCATTAGGCA	GACATTTTTGTGGCTGCGGA	300
	CGATATGGTCGTCTCCGCAG	CCCGGTTCTGTACAATGGCA	345
Ethylene-responsive transcription factor (ERF)	GTCATAGCGACTGCGATTCT	GGCAGGTTGAGATCGAAAGA	106
Actin (Tanaka 2009)	TGTTAGCAACTGGGATGATATGG	GGATAGCACAGCCTGAATAGC	199
	GTCCTTCTTACCGAGGCACC	TCCCGCTCAGCAGTAGTAGT	314
Cytochrome c oxidase (Cox) - Kyndt et al. (2015)	CTTCTGCTCAATGGCGGGT	CTTCATCGGCAACAACACTGA	200
	TCGGAAATGGGAGCAGAACC	CAGGAAGCACAGCCCTACTC	160
	TGAAGTGATCAACGACCGCA	AACACAATCACCCCAGCACT	311
ADP-ribosylation factor (ARF) (Nabemoto et al. 2016)	TGTGGGATGTTGGTGGTCAG	CCTTCTCCAGATGTGGCACA	301
	GGCTAGGGATGAGCTTCACC	CCTTCTCCAGATGTGGCACA	189

Appendix F – Conference papers: Abstracts

ACTA HORTICULTURAE

VIII INTERNATIONAL POSTHARVEST SYMPOSIUM: Enhancing Supply Chain and Consumer Benefits - Ethical and Technological Issues

Cartagena, Spain, 21 – 24 June, 2016 - Oral presentation: *paper accepted for publication in Acta Horticulturae*

Physiological and biochemical dynamics of sweet potato as affected by controlled atmosphere storage treatments

S. Sowe, S. Landahl, R. Tosetti, M. Anastasiadi and L. A. Terry

Plant Science Laboratory, Cranfield University, Bedfordshire, MK43 0AL, United Kingdom

Abstract

The effects of controlled atmosphere on sweet potato are less reported compared to tuber crops such as potato. There is a paucity of information on the role that CA storage plays in controlling the physiological deterioration and changes in non-structural carbohydrates in sweet potato during storage. The overall objective of this study is to elucidate the efficacy of CA treatments on the physiological and biochemical (non-structural carbohydrates) changes of sweet potato during storage. The root samples from a single cultivar were stored in 16 L airtight boxes at 20˚ C. The root samples in the boxes were then flushed with gas treatments of 5 kPa CO2 and 8 kPa O2 integrated with and without 0.001 kPa exogenous ethylene. Respiration rates were significantly different among all treatments ($p<0.05$) and significantly decreased with storage duration. The mean weight loss of marketable roots among the respective treatments was also significantly different. Similarly sweet potatoes stored in CA combined with ethylene produced a significantly higher respiration rate in comparison to those stored in air and CA during the six weeks storage. Sucrose content was significantly higher than fructose, glucose and maltose in all the sweet potato samples irrespective of storage treatment. Results in this study suggest that CA could be a viable option in extending

the post-harvest life of sweet potato during storage under tropical and sub-tropical temperatures.

Keywords: *Ipomoea batatas*, sugars, weight loss, respiration rates

FRUTIC CONFERENCE

Berlin, Germany, 6 – 9 February, 2017 - Oral presentation

Fungal development and potential mycotoxins on sweet potato stored under controlled atmosphere and ethylene conditions

S. Sowe[a], A. Medina[b], R. Tosetti[a], S. Landahl[a] and L. A. Terry[a]

[a]Plant Science Laboratory and [b]Applied Mycology Group, Cranfield University, Bedfordshire, MK43 0AL, United Kingdom

Abstract

The roots of sweet potato are susceptible to rapid postharvest bio-deterioration such as rotting; these are due to fungal and bacterial activity which can be exacerbated during storage. The control of fungal development in sweet potato during storage is important because the pathogens affect the roots aesthetic quality, storage life and nutritional value and may produce mycotoxins which in turn represent a potential hazard to human and animal health. In an attempt to unravel the mechanisms of fungal development, disease incidence and associated mycotoxins production during storage of sweet potato, this study critically evaluates three storage treatments: continuous CA (8 kPa O2 and 5 kPa CO2), Air (0.003 kPa CO2 and 21 kPa O2), and CA supplemented with 0.001 kPa ethylene, over a period of 12 weeks at 20˚C using an orange-fleshed cultivar (06-52). Sampling was done before storage and every six weeks during storage. Three square centimetre of root tissue were extracted from the proximal, middle and distal parts of the root and then mechanically mashed with 75mL of sterile water. Serial dilution plating in Potato Dextrose agar (PDA) was used to evaluate fungal development and isolated fungal spores were sub-cultured in Yeast Extract Sucrose agar (YES) and subsequently analysed for mycotoxins using a HPLC system (Agilent, UK).

Results indicated that sweet potatoes stored in ethylene integrated CA and air exhibited a significant increase in fungal development and consequent decay. Significant differences across the proximal, middle and distal parts of the roots were observed across all the storage treatments ($p<0.05$). Soft and surface rots were predominant at the proximal and distal ends of the root whereas the middle part revealed the least number of fungal growths and rot incidence irrespective of the storage treatment. There was a significant increase in fungal activity by the end of storage for sweet potatoes stored in ethylene integrated CA across all locations; no such increase was observed in the case for those stored in continuous CA. Also storage in air resulted in an increased fungal development at the middle parts of the roots with concurrent decrease at the proximal and distal parts. Storage of sweet potatoes in continuous CA appears to be effective in mitigating fungal growth development. Also sweet potatoes treated with CA were resistant to attack by Aspergillus species and hence were free of contamination by Aflatoxins (B1, B2 and G1). Storage of sweet potatoes in conditions of elevated CO2 levels and sub-tropical temperatures could be useful in designing effective control strategies for Aflatoxin in the tropics.

Keywords: *Ipomoea batatas,* disease, Aflatoxin, *Fusarium, Penicillium, Aspergillus,* ethylene