CRANFIELD UNIVERSITY

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PHYSIOLOGICAL AND BIOCHEMICAL CHANGES IN POTATO STOCKS WITH DIFFERENT SUSCEPTIBILITY TO BLACKHEART DISORDER

School of Energy, Environment and Agrifood (SEEA)

Plant Science Laboratory

PhD THESIS

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Supervisor: Professor Leon A. Terry

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for the degree of Doctor of Philosophy

‘’Whatever you do in life will be insignificant, but it's very important that you do it 'cause nobody else will...’’

~ Mahatma Gandhi ~

ABSTRACT

Blackheart (BH) is a non-pathogenic internal physiological disorder induced in potato tubers during storage. It is usually associated with oxygen (O_2) depletion and/or carbon dioxide (CO_2) accumulation. BH symptoms are characterized by a dark brown to black discoloration in the central portion of the tuber tissues. It is believed that phenolic compounds are related to tuber tissue discoloration and development of BH. In recent years, this disorder has been a particular problem for the UK fresh and packed potato industry and to date there is still no true understanding of the causal factors that govern BH susceptibility in stored potato tubers. Accordingly, the aim of this project was to elucidate the physiological and metabolomic mechanisms involved in potato blackheart disorder and to have a better understanding of the factors which contribute to BH development in order to alleviate this problem for the UK fresh potato industry.

The symptoms of BH may be absent in potato tubers during storage and become more evident after washing, conditioning and packing, and thus only during the subsequent shelf-life and home-life period. Physiological and biochemical analysis of potato stocks cv. Maris Piper with different susceptibility to BH (two susceptible to BH stocks and one non-susceptible) was performed. All Maris Piper stocks used were grown in different locations and growing conditions. In 2011-2013, an attempt to mimic the shelf life conditions was evaluated by initially storing potato tubers at very low temperatures (1.5 or 3^{o}C) and then transferring them to air (21% O_2) and/or various O_2 and CO_2 concentrations using a CA system at 15 or 20^{o}C for shelf-life evaluation. In 2013-2014, micro-Computed Tomography scanning was used for better visualization of the intercellular spaces of tuber tissues. The O_2 diffusion in flesh and heart tuber tissues was measured using a gas diffusion measurement model set up coupled with optical sensors for the first time. Four experiments were conducted

over three years (2011-2014). This study showed that the cold initial storage temperature was the main factor affecting both physiological and biochemical changes in potato tubers with different susceptibility to BH. Respiration rate recorded at 15^{o}C was greater for those tubers that had been held under 10% CO_2 and lower when tubers were stored at 5% O_2 at 20^{o}C due to CO_2 efflux and O_2 depletion respectively. However, a relationship between respiration rate and the incidence of BH was not found as it was expected. The BH incidence was quite low proportional to the number of potato tubers used in this project where less and more intense brown discolorations were observed in the tuber heart tissues of these susceptible stocks to BH. BH incidence was greater in those tubers stored in air (21% O_2) compared to those that have been held under various gas combinations. Also, it was unclear whether those discolorations were stimulated or induced at very low initial storage temperature and then exacerbated during shelf-life evaluation at either 15 or 20^{o}C. The temperature and exposure period in which BH showed greatest incidence could not readily be predicted. Biochemical analysis revealed that sugars tended to be more accumulated in those samples of stocks with BH susceptibility and that might be an indicator of stock susceptibility. Chlorogenic acid and isomers thereof and specific amino acids (tyrosine, phenylalanine and tryptophan) tended to be more accumulated in tuber heart tissue samples derived from stocks with increased susceptibility to BH. However, it is still unclear whether tyrosine or chlorogenic acid or other phenolic compounds that were not quantified contributed to these brown tissue discolorations found in this project. Furthermore, untargeted metabolomic analysis on selected flesh and heart samples showed a plethora of known and unknown metabolites. Significant differences in the metabolome regulation were shown between discoloured and control samples. Yet, the presence of glycoalkaloid and flavonoid content in those samples of stocks with no susceptibility to BH might suggest differences in gene expression under the storage conditions studied and could be indicators of potato stock susceptibility or possible indicators of geographical provenance of potato stocks cv. Maris Piper.

Also, in 2013-2014 both micro-CT scans and 3D images showed a variation in the structure and connectivity of the intercellular spaces in tuber tissues. A large variation in O_2 diffusivities was shown and it was suggested that this might be due to the discontinuity of the gas-filled intercellular spaces. Due to the large variability, no differences between flesh and heart tissue were observed. Furthermore, the fact that there were no indications of tissue discoloration in these samples susceptible to BH, a comparison in stock susceptibility was not possible.

It is still difficult to explain the real factors that govern the BH disorder development and the symptoms that differentiate the disorder from similar internal physiological disorders induced in potato tubers. Further physiological and biochemical research is required in order to confirm whether those findings are related with BH disorder.

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LIST OF ABBREVIATIONS

Δp	partial pressure difference
Δt	time difference
µm	microgram
µl	microliter
1HNMR	Proton Nuclear Magnetic Resonance
3D	three-dimensional
ANOVA	Analysis of Variance
BC	brown centre
BCL	brown centre light
BH	blackheart or dark to black (for discoloration)
BL	baseline
BP	barometric pressure
^{o}C	Celsius degrees
Ca	Calcium
CA	Controlled Atmosphere
ca.	approximately
cef	compound exchange formatted
CIS	cold-induced sweetening
cm	centimetre
CO_2	carbon dioxide
CT	Computed Tomography
cv(s).	cultivar(s)
D_{O2}	oxygen diffusion
DW	Dry Weight
ECC	Extracted Compound Chromatogram
e.g.	for example
ELSD	Evaporative Light Scattering Detector
et al.	and others
FC	fold change
FD	Freeze Dried
FIE-MS	Flow Injection Electrospray Ionization-Mass Spectrometry

Fig.	figure
FPSA	*Fresh Potato Suppliers Association*
FR	flow rate
FW	Fresh Weight
g	gram
GLM	General Linear Model
GC	Gas Chromatography
h	hour
HCOOH	formic acid
HH	hollow heart
HPLC	High Performance Liquid Chromatography
HPLC-UV	High Performance Liquid Chromatography with Ultraviolet
IBS	internal brown spot
i.e.	that is
IHN	internal heat necrosis
K	potassium
kg	kilogram
kPa	kilopascal
L	litre
LC	Liquid Chromatography
log10	logarithm base 10
LSD	least significant differences
LTS	low temperature sweetening
m	meter
m/z	mass-to-charge ratio
MAP	Modified Atmosphere
MeOH	methanol
mg	milligram
min	minute
ml	millilitre
mm	millimetre
MPP	Mass Profiler Professional
MS	Mass Spectrometry
MS/MS	tandem mass spectrometry

n	number
N_2	nitrogen
ng	nanogram
NMR	Nuclear Magnetic Resonance
O_2	oxygen
OT	outturn
%	percent
P	probability
p	partial pressure
PAL	phenylalanine ammonia lyase
PCA	Principal Component Analysis
POD	peroxidase
PPO	polyphenol oxidase
PSL	Plant Science Laboratory
PVC	polyvinyl chloride
QC	Quality Control
QTL	quantitative trait loci
R	universal gas constant
R_{CO2}	carbon dioxide production
R_{O2}	oxygen consumption
ROS	reactive oxygen species
rpm	rotations per minute
s or sec	second
SBCSR	Sutton Bridge Crop Science Research
T	temperature
TIC	total ion chromatogram
TOF	Time-of-flight
V	volume
v/v	volume per volume
viz.	that is
WVP	Water vapour pressure

CHAPTER ONE:
GENERAL INTRODUCTION

1.1. Project background

The potato crop (*Solanum tuberosum* L.) is considered the most important member of the nightshades family (Solanaceae) and currently ranks sixth around the world and fifth in the UK (FAO, 2013). Potato is a high value crop supplying dietary energy and is widely produced and consumed in the UK. However, its quality can be influenced, under adverse environmental and undesirable storage conditions, leading to physical and chemical quality losses affecting consumer acceptability and subsequently economic losses. Potato tubers may be susceptible to more than 100 external or internal diseases and disorders. The internal physiological disorders in potatoes are mainly related to tuber tissue discoloration resulting in cell necrosis (Sowokinos, 2007).

An internal disorder named blackheart was initially observed and reported in the Northern USA when shipping or transporting potato tubers (Bartholomew, 1916). Blackheart is a non-pathogenic disorder resulting in an internal brown to black discolouration (normal tuber flesh colour to pink, brown and finally black) of mainly medullary tuber tissues (pith) and rarely cavity formation (Davis, 1928). Kumar Chaurasia (2009) characterized the symptoms as a dark grey to purplish discoloration. The discoloration may be irregular in shape or more circle-like when induced by air exclusion or exposure to high temperatures, respectively (Stewart and Mix, 1917). Sometimes blackheart may diffuse into the unaffected perimedulla area without reaching the cortex of the tuber (Hooker, 1981). The affected flesh tissue remains firm and odourless and no external symptoms are apparent, thus an intact but affected tuber looks healthy. It has been hypothesized that phenolic compounds such as

chlorogenic acid and derivatives and tyrosine causing tissue discoloration may possibly be linked with blackheart development (Bartholomew, 1916; Reeve, 1968). In 1980, Jadhav *et al.* reported that tubers with slight blackheart incidence contained higher glycoalkaloid content with this being increased when severe blackheart symptoms were observed. It has also been reported that blackheart may either be developed by tuber exposure to high temperatures (Bartholomew, 1916; Davis, 1928; Kumar Chaurasia, 2009) or at low or very low temperatures (Stewart and Mix, 1917; Lipton, 1967; Hooker, 1981).

Blackheart is usually associated with a lack of oxygen (O_2) and/or carbon dioxide (CO_2) accumulation (Davis, 1928; Hooker, 1981). It has been hypothesized that when O_2 supply is depleted and is unable to reach the tissue internally, blackheart is induced. Furthermore, at extremely high or low temperatures the disorder may be developed as result of delayed gas diffusion within tissue, thus CO_2 is formed and more rapidly accumulated in the internal atmosphere. According to Lipton (1967), storing potato tubers at 15-20^{o}C in 0.5-1% O_2 blackheart being induced. Potato tubers held at under 10% CO_2 also showed incidence (Butchbaker *et al.*, 1967). The study of the intercellular space in potato tuber and gas diffusion through it has been of interest since the late 1890's until the late 1990's. In 1988, Banks and Kays proposed that further research on tuber resistance to O_2 diffusion is needed in order to investigate the disorder. However there has been a paucity of research since then. Still there remains no true understanding of the causal factors that govern blackheart susceptibility in potato tubers.

This disorder is a particular problem for the UK fresh and packed potato industry. Customer complaints due to blackheart disorder mainly start in January, peaking in May, June and July. Blackheart affected tubers look normal externally and thus can easily pass the quality control procedures undermining consumer confidence later. Among those potato cultivars produced in the UK, it was reported that Maris Piper which is the dominant main-

crop produced (accounting for 15% of UK plant area) has the greatest susceptibility to blackheart disorder followed by cvs. Marfona, Estima and Vales Sovereign.

1.2. Aim and objectives

1.2.1. Aim

The overall aim of this project was to elucidate the physiological and metabolomic mechanisms involved in potato blackheart disorder and to have a better understanding of the factors which contribute to the development of blackheart disorder in order to alleviate this problem for the UK fresh potato industry.

1.2.2. Objectives

1. to examine the effect of different storage conditions on respiration rate of tubers from potato stocks cv. Maris Piper with different susceptibility to BH and the possible link with the incidence of blackheart disorder
2. to identify compounds related or involved with tuber tissue discoloration
3. to determine the basis and temporal change of gaseous permeability of potato tissue in relation to blackheart disorder.

1.3. Thesis structure

Nine chapters in total comprise this PhD thesis. The general introduction is presented in this Chapter One. Chapter Two includes the literature review and Chapter Three, the Materials and Methods of all four large scale experiments conducted at Cranfield University throughout this PhD project. Results of experiment 1 conducted in year 1 (2011-2012) are

presented in Chapter Four, while Chapter Five includes the results of both experiments conducted in year 2 (2012-2013). Untargeted metabolomics analysis applied on selected samples from year 1 and also samples derived from the 1st experiment in year 2 is presented in Chapter Six. Chapter Seven represents the gas diffusivity experiment which was carried out in year 3 (2013-2014) at the Department of Metallurgy and Materials Engineering (MTM/MME) and the Faculty of Bio-science Engineering (K.U.Leuven, Belgium). In Chapter Eight the general discussion and the conclusions are presented, while the literature cited is provided in Chapter Nine. Appendix A includes example images of discoloured potato tubers from both years 1 and 2. All statistical analysis tables from experiments in year 1 are presented in Appendix B corresponding to Chapter Four. Appendix C includes all statistical analysis tables from both year 2 experiments which correspond with Chapter Five. In Appendix D and E, example figures of total ion chromatograms (TIC) and all ANOVA and fold-change analysis tables of untargeted metabolomics analysis applied in year 1 and year 2, respectively, are included corresponding to Chapter Six.

Results from this project have already been presented at the following International Conferences:

Elisavet Kiaitsi, Leon A. Terry. Storage temperature and simulated shelf-life conditions influence respiration rate and incidence of blackheart disorder in potatoes. *XI International Controlled & Modified Atmosphere Research Conference*, Trani, Italy, 3-7 June 2013 (oral presentation).

José Juan Ordaz Ortiz, **Elisavet Kiaitsi**, Leon A. Terry. Discovery, identification and metabolite variation in potato tubers with blackheart symptoms: taking metabolomics into postharvest. *VI International Conference on Managing Quality in Chains (MQUIC),* Cranfield, England, U.K., 2-5 September 2013 (oral presentation).

1.4. Declaration

Blackheart disorder development was conducted using a severe method at Sutton Bridge Crop Storage Research (SBCSR, UK) by Adrian Briddon in order that susceptible and non-susceptible blackheart potato stocks cv. Maris Piper could be selected and transferred to Cranfield University (UK) during all three years of this project (Chapter 3; Section 3.2 and 3.7). Phenolic content analysis and untargeted metabolomics analysis (Chapter 3; Section 3.6) using the Liquid Chromatography Mass Spectrometry (LC/MS) instrument was carried out under the supervision of Dr. Jose Juan Ordaz Ortiz. All statistical analysis was conducted with the assistance from the statistician Dr. Patricia Bellamy.

CHAPTER TWO:
LITERATURE REVIEW

2.1. Introduction

It is believed that losses during potato storage in the UK are around 3 – 5%, the main causes of which are detailed in Figure 2.1 (Terry *et al.*, 2011). Blackheart disorder (BH) has been identified as a significant cause of waste in the Great Britain (GB) potato industry; however, its impact is probably under-estimated since it tends to only manifest itself during shelf-life and home-life. There is no reliable data on the incidence of blackheart in the GB potato industry, yet there is consensus amongst industry leaders that the disorder is a growing problem and one that should be addressed in order to safeguard against possible product displacement by imports.

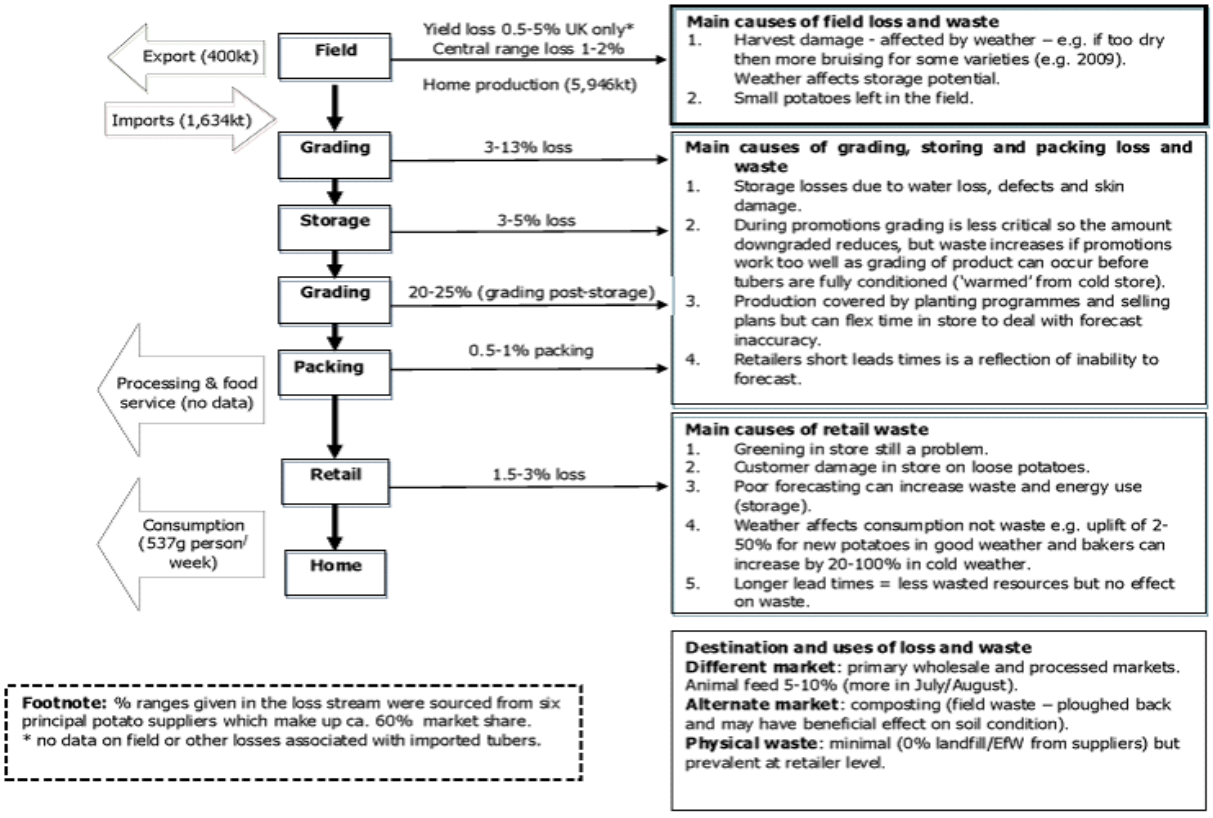


Figure 2.1 Resource map of UK potato yield losses (Terry *et al.*, 2011 – more information available on http://www.wrap.org.uk/retail_supply_chain/research_tools/research/report_resource.html).

2.2. The blackheart disorder

Blackheart is a physiological non-pathogenic disorder resulting in an internal brown to black discolouration indicated mainly in the medullary tuber tissues (pith) and rarely cavity formation (Fig. 2.2 a, b and c) associated with O_2 depletion and/or CO_2 accumulation. This physiological disorder was initially introduced by Bartholomew in 1916 when the author observed dark brown or black tissue discolorations in potato tubers during shipment and transport. Bartholomew believed that BH is developed due to overheating at shipping and suggested that exposure to high temperatures < 35^{o}C cause changes in respiration rate resulting in cell suffocation due to high levels of O_2 demanded, CO_2 accumulation in the interior of the central tuber tissues and eventually death (Bartholomew, 1916). A few years later, Stewart and Mix (1917) after a three year study on BH based on Bartholomew's observations they concluded that BH disorder may be induced at any temperature by O_2 depletion as was able to be developed by storing tubers in hermetically sealed jars after 20 days at 12-15^{o}C. Furthermore, they showed that tuber size was not necessarily an important factor contributing in BH susceptibility.

In those cases, when O_2 supply is depleted and is unable to reach the tissue internally, blackheart is induced. Furthermore, at extremely high or low temperatures the disorder may develop as a result of delayed gas diffusion within tissue, with CO_2 accumulating more rapidly in the internal atmosphere. To date it is still believed that BH may either be developed by tuber exposure to high temperatures (> 35^{o}C) without O_2 deprivation (Hiller, 2002; Kumar Chaurasia, 2009) or at low or very low temperatures (Link *et al.*, 1932; Lipton, 1967; O'Brien and Rich, 1976; Smith; 1978; Hooker, 1981; Wale *et al.*, 2008). According to Lipton (1967), storing potato tubers cv. White Rose at 15-20^{o}C in 0.5-1% O_2 induced blackheart disorder. Potato tubers cv. Kennebec held under 10% CO_2 also showed incidence (Butchbaker *et al.*, 1967).

The disorder poses a particular problem for the GB potato industry where a large proportion of the crop is marketed from store. Symptoms may also be absent in crops throughout storage and only become apparent after washing, conditioning and packing. However, during the subsequent shelf-life and home-life period BH becomes evident. Blackheart is a particular problem for the fresh potato industry because quality control (QC) procedures cannot adequately check for the defect. That is, they cannot adequately predict which consignment will be at risk. This failure is undermining consumer confidence. It is believed that blackheart does not cause any nutritional losses to potato tubers but it is estimated to account for 25-30% of consumer complaints (David Walker, Chairman FPSA, personal communication) due to it being aesthetically unappealing. BH does not show external symptoms and only becomes apparent when tubers are sliced open. Thus tubers look superficially healthy from the first point of view. Upon cutting brown or black tissue discoloration is evident (severity depended), but sometimes a longer time is required for the discoloration to progress (normal tuber flesh colour to pink, brown and finally black discoloration) (Davies, 1928; O'Brien and Rich, 1976). Therefore, it seems that BH discoloration symptoms vary and may be formed irregularly or more circle-like shape when induced by air exclusion or exposure to high temperatures respectively (Stewart and Mix, 1917). Kumar Chaurasia (2009) characterized the symptoms as a dark grey, black or purplish discoloration at high temperature exposure. Sometimes it may diffuse into the unaffected perimedulla area without reaching the cortex of the tuber (Hooker, 1981). However, it is unknown at which temperature and exposure time BH is initiated and reaches maximum levels of incidence. Sowokinos (2007) has pointed out that internal physiological disorders might be slowly expressed. Due to brown or black tissue discoloration (severity depended) sometimes the disorder is mistakenly referred to as incipient hollow heart, brown centre, brown heart or sugar heart (Bussan, 2007), and this diversity in nomenclature has led to some

confusion over identifying any underlying causal factors which may promote the specific disorder (Link *et al.*, 1932; Wolcott and Ellis, 1956, 1959; Reeve, 1968; Sowokinos, 2007).

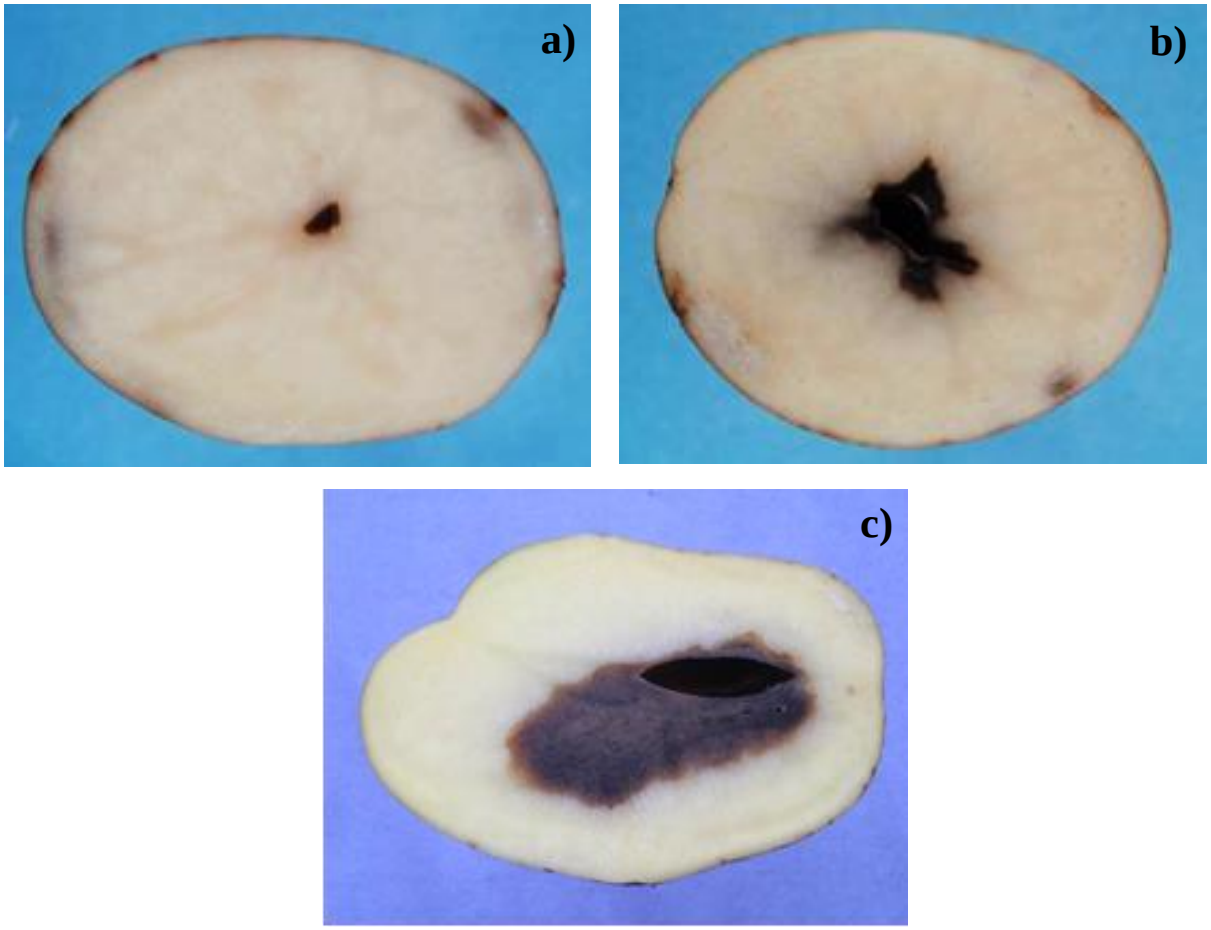


Figure 2.2 Potato tubers with symptoms of blackheart showing discolouration and cavitation in central tissues (a, b and c) (source A. Briddon).

It is more likely that BH is induced during storage and does not tend to occur in crops from the field; however, it has been suggested that physiological disorders and tissue discolorations may be initiated at pre-harvest conditions such as growth conditions, soil temperature and type (silts) and water logging (flooding) (Link *et al.*, 1932; O'Brien and Rich, 1976; Bussan, 2007; Wale *et al.*, 2008; Palta, 2010; Zommic *et al.*, 2013). Other factors have been highlighted as being potentially linked to blackheart and other internal browning disorders. These include tuber maturity, drought stress, high temperatures, large tuber size

and the use of some plastic packaging films (Larson and Albert, 1945; Walcott and Ellis, 1959), but none of these causal factors reliably affect blackheart incidence. There is still no true consensus on the causal factors which makes one particular tuber stock more or less susceptible than another. Although seasonal variations are observed, the incidence of blackheart is reported to have increased in the last five years (David Walker, Chairman FPSA, personal communication). It is believed that the increased efficacy in logging consumer complaints does not fully account for this apparent increase, such that a change or combination of changes has occurred in the last five years that may be responsible for increased incidence of the disorder. Some candidates persist, for example, the increased usage of plastic packaging. Yet, it is likely that a combination of factors is responsible.

To reiterate, blackheart is a particular problem for the fresh, packaged potato industry, because typically only central tissues become necrotic and tubers appear otherwise healthy. Crops may pass QC checks and be marketed with defects only becoming apparent to the consumer after preparation during cooking (e.g. baked potatoes). Customer complaints from blackheart have increased in recent years. FPSA estimate that currently there are approximately 2,500 consumer complaints annually (D. Walker, personal communication) and it is likely that this level of dissatisfaction is much higher, but not reported. Customer complaints can start in January and peak in May, June and July. Some supermarkets have indicated that crops of critical cultivars in GB, for instance Maris Piper, which is the dominant main-crop produced (accounting for 15% of UK plant area), will be sourced from abroad if the incidence of blackheart cannot be substantially reduced.

2.2.1. Biochemistry basis of the disorder

Blackheart manifests itself as a darkening of central tissues in the tuber (Figure 2.2). However, the colour change can be progressive from light reddish or brown with areas mainly confined to the central pith. Indeed, these smaller areas are not always evident immediately after cutting (Reeve, 1968). The brown to black coloration indicates a phenolic-based reaction. Phenolics are any compound which contain an aromatic carbon ring and associated hydroxyl group and thus include phenolics acids and flavonols within the phenylpropanoid pathway (Vogt, 2010).

Initially, Bartholomew (1916) suggested that black discoloration is caused by tyrosine oxidation via polyphenol oxidase (PPO or tyrosinase). Later, Reeve (1968) first demonstrated that cells surrounding affected tissue could be highly stained with Sudan IV in histological studies, indicating the presence of suberin and other phenolics. Additional histochemical tests for both chlorogenic acid and tyrosine showed more intensive positive colour reaction in blackheart affected tissue than did normal healthy parenchyma tissue from the central region in unaffected tubers. Unfortunately, there is no information which is publically available which has attempted to show there to be a correlation between other phenolics found in potato (*viz*. caffeic acid, p-coumaric acid, ferulic acid and trans-cinnamic acid; Yao *et al*., 1995; Mattila and Hellstrom, 2007; Im *et al*., 2008; Andre *et al*., 2009) and blackheart.

It is not clear whether chlorogenic acid, other phenolics (known and unknown) and/or tryptophan are indicators of blackheart or are produced as a result of blackheart. It is thought that tryptophan may affect phenolics accumulation (Yao *et al*., 1995), but whether this is significant is unclear. That is, it is still not known whether tryptophan or phenolics are the cause or effect of the disorder. It is possible that phenylalanine ammonia lyase (PAL) the enzyme to the phenylpropanoid pathway (Gerasimova *et al.*, 2005; Payyavula *et al.*, 2013)

may be regulated following gaseous induced stress (Joos and Hahlbrock, 1991; Geigenberger *et al.*, 2000, 2003). Even though chlorogenic acid is the dominant phenolic found in potato (Dao and Friedman, 1992; Friedman. 1997; Brown *et al.*, 2005; Navarre *et al.*, 2011, 2013) this does not translate into it having a mechanistic role in blackheart. It is likely that other compounds (e.g. plant growth regulators) are involved, since these are up or down regulated according to storage stress and physiological age (storage life).

Blackheart tends to only manifest itself at certain times of the year, especially after longer term storage and conditioning, and can be exacerbated by inappropriate packaging. Thus, distinct chemical changes must be taking place during postharvest life, which make some tubers more susceptible to the disorder. Given that blackheart tends to only manifest itself from January onwards, it is probably that the transition from endodormancy (true dormancy) to ecodormancy (sprout suppression phase) may be significant, and this might point to a role for plant growth regulators and their metabolites as these are known to flux during storage. Targeted and non-targeted metabolomics might provide an insight and ultimately a greater mechanistic understanding of blackheart allowing for presymptomatic risk assessment by establishing predictive biomarkers. To date, no detailed metabolomics have been conducted.

2.3. Gaseous diffusion and role of packaging

It has long been recognised that insufficient gas exchange between tubers and the external atmosphere is associated with blackheart. Gas exchange is caused by differences in gas composition between the applied external atmosphere and the internal atmosphere due to O_2 consumption and CO_2 production during respiration. The latter phenomenon is caused by overall gradients which may develop due to the large difference in CO_2 and O_2 diffusivity during inappropriate modified atmosphere packaging (MAP). In contrast to other crops which

suffer from internal storage disorders (e.g. core breakdown in controlled atmosphere stored conference pears, Ho *et al*., 2006) no recent research has been conducted on understanding gaseous diffusion in potato tissue.

It has been proposed that where gaseous exchange cannot keep pace with increased respiration rate (e.g. during conditioning) then blackheart incidence will increase (Davis, 1928). High respiration is associated with higher temperatures. Bartholomew (1916) believed that high temperatures resulted in an accumulation of CO_2 and a lack of O_2 in the central tissues. He believed that higher temperatures would act upon the enzyme PPO, and that greater enzyme activity would produce black melanic substances. He showed that he could induce the disorder by holding tubers between 38-44^{o}C for 15-24 hours. However, high temperature is not a necessary precondition for blackheart to occur. Indeed, Stewart and Mix (1917) where able to induce blackheart by storing tubers in hermetically sealed jars for about 20 days at 12-15^{o}C. Later, Davis (1926) showed that blackheart could be induced by holding tubers at 45^{o}C in a carbon dioxide free atmosphere with abundant oxygen available. He showed that during the time preceding the appearance of the disorder, the internal level of CO_2 rose to 50% whilst O_2 was reduced to 4%. What can be noted is that the work by Bartholomew, Stewart and Mix, and Davis is nearly 100 years old. This does not mean it is any less valid, but was conducted on older varieties grown predominantly in the USA, and clearly did not benefit from the recent advances in genomics and metabolomics. It was only with the pioneering work of Kidd (1919) and Barker (1936) and then subsequently by Burton and colleagues between 1950 and 1970 (Burton, 1958; 1962) that research focussed once more on elucidating the effects of gaseous composition on tuber physiology. However, in the main, this more recent work was centred on extending storage life (i.e. sprout suppression) without increasing sugar accumulation in processing varieties. For instance, Khanbari and Thompson (1994) cured potato tubers cv. Record for three weeks at 10°C before being

transferred to controlled atmosphere storage at 4°C for six months. Concentrations of 0.7 – 1.8% CO_2 in combination with low O_2 (2.1-3.9%) gave the best results with light crisp colour, low sprout growth and few rotted tubers compared with 0.9% CO_2 and 21% O_2. Burton (1959) found that increasing CO_2 concentration was negatively correlated with sprout growth where levels as high as 20% CO_2 completely eliminated sprout growth after 4 months at 10°C. This was confirmed many years later by Khanbari and Thompson, (1994) who found higher CO_2 resulted in better sprout inhibition, however fry colour became darker. Only, Lipton (1967) has provided evidence on the effects of gaseous composition on blackheart. He showed that cv. White Rose tubers developed blackheart if they were held at 1 and 0.5% O_2. No more information was available on defining gaseous compositions which encourage blackheart.

Early work by Burton (1965) investigated the amount of dissolved gases in the cell sap of tubers and found that the optimum CO_2 concentration for growth to be 2-4 % or 0.04-0.05 ml CO_2 ml^{-1} sap whereas inhibition of growth was achieved at much higher CO_2 concentrations. The author also found that low O_2 stimulated growth especially around 5% which equates to 0.0006 ml O_2 ml^{-1} sap. It was concluded that since temperature affects the solubility of gases, increasing the storage temperature above 10°C in an air atmosphere would increase the amount of dissolved gases in the cell sap and the resulting sprout growth may be no more than would be expected as a result of the increased CO_2 in solution. Even though this work was not done in the context of blackheart, it remains the only piece of work that has systematically detailed gas exchange in potatoes.

Despite indications that packaging may influence the incidence and severity of blackheart, there is no literature in the public domain which has evaluated the effect and mechanisms involved. Only one piece of work has researched the effect of packaging on tuber composition (Gosselin and Mondy, 1989) and this was not focussed on blackheart.

However, they did report that cvs. Russet Burbank and Chieftain packaged in polyethylene and held at 20^{o}C had lowest weight loss, ascorbic acid and nitrate-nitrogen but highest in discoloration, phenols and glycoalkaloids than those packaged in mesh or paper. Potatoes packaged in paper had the lowest discoloration and phenols and highest ascorbic acid. Anecdotally, it is believed that loose tubers suffer less risk of blackheart than tubers packaged in MAP. Ironically, it may be that inappropriate MAP may inhibit sprouting but encourage blackheart.

2.4. Other internal physiological disorders in potato tubers

Along with BH disorder, similar internal physiological disorders resulting in tissue discoloration incidence are induced in potato tubers that pose a problem to both fresh and processing markets. Internal physiological disorders share a common feature which is that of the brown or black tissue discoloration localized either in the central part of the tuber (pith) or randomly in the perimedulla zone as a result of cell membrane damage. Most of those physiological disorders are developed due to adverse environmental and growing conditions. Sometimes these disorders may overlap, thus difficulties in diagnose are caused due to similar evidence of initial or severe symptoms indicated internally in the tuber tissues (Reeve, 1968; Wale *et al.,* 2008). It would be useful to mention some of those disorders that might coexist with BH or act as precursors. Brown Centre (BC) which may also referred as sugar heart or brown heart is characterized by brown tissue discoloration with necrotic lesions in the central pith part of the tuber. It has been reported that this disorder may be caused at pre-harvest period due to stressful growth conditions such as cool temperature soils (<10 – 15^{o}C) and nutrient deficiency mainly calcium (Ca^{2++}) and potassium (K) (Van Denburgh *et al.,* 1980, 1986; Davies, 1998; Bussan, 2007; Sowokinos, 2007, Palta, 2010). Calcium has a crucial role in plant growth maintaining the cell membrane integrity and providing cell wall

strength. Palta (2010) reported that efficient calcium pre-harvest may reduce the incidence of internal physiological disorders. An alternative but similar to that of BH induction hypothesis has been proposed for BC where limited supply of O_2 and/or CO_2 accumulation internally in the tissue leads to cell damage and maybe death; however, more research is needed to that hypothesis. BC may be the initiator of hollow heart (HH), another physiological disorder resulting in cavities formed up to 2 or more cm in diameter in the pith tuber area and may be accompanied with brown tissue discoloration around the cavities (Levitt and Minn, 1942; Gunter, 2002; Bussan, 2007; Elbatawi, 2008). Larger tubers may be more prone to HH caused by rapid tuber bulk, but small tubers may also be affected where osmoregulation may be disturbed. Thus the perimedulla tuber area is rapidly enlarged causing a separation in the pith later leading to HH formation (Crumbly *et al.*, 1973; Mogen and Nelson, 1986; Rex and Mazza, 1989; Elbatawi, 2008). Tai and Misener (1994) have previously pointed out that the narrowness of the pith is positively associated with the tuber length and longer tubers have narrow pith and pith cells localized in various tuber parts (namely central, stem and dub end) may vary in size (Reeve *et al.*, 1971; Mogen and Nelson, 1986). Another disorder which has been studied in more detail is the internal brown spot (IBS) or internal- browning, rust spot, brown fleck, necrosis or chocolate spot. IBS is characterized by irregular brown or rust coloured spots or blotches indicated mainly in the perimedulla zone (Reeve, 1968; Davies and Monk-Talbot, 1990; Davies, 1998; Sowokinos, 2007; Hiller and Thornton, 2008; Vanoli *et al.*, 2012). IBS often overlaps with internal heat necrosis (IHN) due to similar symptoms induced internally of the potato tubers (Sterrett and Henninger, 1997).

2.5. Metabolomic approaches on potatoes

2.5.1. Introduction

The scientific field of ‘’-omics’’ refers to the functional genomics (gene expression) and the importance of integrating molecular data combined with the analysis of transcriptomics (mRNA), proteomics (proteins) and metabolomics (metabolites) (Dunn and Ellis, 2005; Vorst *et al.*, 2005, Shepherd *et al.*, 2011). The parallel analysis of mRNA and proteins as products of gene analysis (Fiehn *et al.*, 2000) has become established. However the metabolites are defined as the missing link of the functional genomic approaches (Hall *et al.*, 2002) since the metabolome represents the final measurable response of an organism.

The metabolite profiling was initially developed in the early 1970’s by the Baylor College of Medicine (Sumner *et al.*, 2002). In the 1980’s, there was a development of analytical techniques applied such as Soft Ionization, GC-MS and NMR and an increase in publications annually which led to the adaption and use of the new technology. However, in terms of the plant system the use of metabolite profiling as a diagnostic technique started in the early 1990’s (Maloney, 2004).

The major path of metabolomics is defined as the comprehensive profiling of all metabolites aiming to identify a much larger possible number of these molecules (Genga *et al.*, 2008) to better understand them in a biological system (Tolstikov, 2003). Metabolomic study is therefore defined as the non-biased quantification of metabolites that provides a better understanding of how an organism responds or a tissue functions. Metabolites are low molecular weight molecules that are presented in a cell and are required for metabolic reactions and the collection of these metabolites is called the metabolome (Dunn and Ellis, 2005). In terms of the size of primary and secondary metabolites with various chemical structures (Genga *et al.*, 2008) present in plants has estimated ca. 50000 and about 200000

metabolites are predicted for the plant kingdom in total and it is estimated that currently metabolite identification amounts ca. to 10% (Allwood *et al.*, 2008; Hall *et al.*, 2002; Alliferis and Jabaji, 2012; Patti *et al.*, 2012). For example, 300 metabolites were found after metabolomics application on *Arabidopsis thaliana* (Fiehn *et al.*, 2000), a plant model which is widely being used for functional genomics (Dunn and Ellis, 2005; Kusano and Saito, 2012). Several steps are required for a metabolomic procedure starting from sample preparation and extraction, instrumental analysis, statistical analysis, metabolite identification and data interpretation.

2.5.2. Metabolomic studies on potatoes

Potatoes have previously been used as a model plant for targeted or untargeted metabolomics analysis. Genetically modified (GM) potatoes have also been extensively examined using metabolite profiling for compositional changes and comparison with conventional potatoes. Defernez *et al.* (2004) examined the effects of genetic modifications on tuber metabolites between 40 GM lines and control samples of 4 groups derived from Record and Desiree potato cultivars by Proton Nuclear Magnetic Resonance (1HNMR) and High Performance Liquid Chromatography with Ultraviolet (HPLC-UV) detection. There were some significant differences between GM lines and their control samples, however, the largest differences were observed between the two cultivars. A similar metabolomics study by Catchopole *et al.* (2005) on GM potatoes cv. Desiree using Flow Injection Electrospray Ionization-Mass Spectrometry (FIE-MS) showed a compositional similarity between GM and traditional potatoes. FIE-MS technology has also been used by Beckmann (2007) for metabolomics analysis applied on potatoes in order to investigate differences of the chemical composition and quality traits of potato cultivars. Carreno-Quintero *et al.* (2012) has also reported an untargeted metabolomics study on quantitative trait loci (QTL). Furthermore,

Vorst *et al.* (2005) reported an untargeted metabolomics analysis applied on potato genotypes featured with highly breeding population using the LC-MS technique.

Additionally, phytochemical diversity of potato genotypes (group Andigena, Phureja, Stenotomum and Tuberosum respectively) cultivars and Chilean landraces were examined by Dobson *et al.* (2007, 2008, and 2010) using a GC-MS based metabolite profiling study. As previously mentioned GC-MS is the most common and mature widely applied analytical technology and has successfully been applied to access changes in metabolites present in potatoes. Roessner *et al.* (2000) developed a method applied on soil-grown and artificially induced potatoes (cv. Desiree) using GC-MS technology for detection and simultaneous analysis of unexpected changes in metabolites. Initially, the method was applied in soil-grown and in vitro potatoes tubers which were extracted in methanol/water solution followed by methoximation and silylation and more than 150 polar compounds -by which 77 with a known chemical structure (amino acids, organic acids, sugars, sugar alcohols and aromatic amines) were identified. In general, this method revealed significant differences in the metabolism among the soil-grown compared to in vitro potato tubers with the latter to contain higher amounts of amino acids and also higher amount of compounds indicative of osmotic stress. Furthermore, unexpected changes in disaccharides and sugar alcohols were revealed after metabolite profiling application in transgenic lines modified with their respect to either sugar or starch metabolism (Roessner, 2000; 2001). Later, Shepherd *et al.* (2007) based on Roessner's method (Roessner, 2000), described the validation of GC-MS approach on the potato metabolite profile by using either Time-of-flight mass spectrometry (GC-TOF-MS) or Quadrupole-mass spectrometry. The method included oximation conditions, identification of metabolites derived from four tissue types (pith, inner and outer cortex and skin) selected from different positions of the tuber (radially and longitudinally slices) selected and a comparison between fresh and freeze-dried (FD) samples. About 180 polar and non-polar

metabolites in total were identified and analysed. Significant differences were observed in both radial and longitudinal tuber slices; however, most metabolites were detected between the different tissue types. A few significant differences were also found for 11 polar and 22 non-polar metabolites between fresh and FD samples. Although the use of FD material provides several advantages such as higher linearity and repeatability; however the author found that both fresh and FD materials are equally applicable for metabolite profiling of cross-sample comparisons.

Another recent metabolomics analysis on potatoes (cv. Desiree) has been reported by Shepherd *et al.* in 2010, where six potato tuber life stages including the effect of storage temperature (5 or 10^{o}C) on mature tubers and also the impact of developing tubers excised from mother plant were examined by using a range of MS approaches. The combination of analytical techniques such as Direct Infusion Electrospray Ionisation (DI-MS), GC-MS and LC-MS was able to identify 161 metabolites (by which 134 showed significant differences) showing differentiate changes in metabolite profiling of tuber life cycle. Moreover, metabolite profiling applied on tubers excised and attached the mother plant respectively, showed the impact of source-sink relations on metabolism and also when applied on stored mature tubers was able to distinguish those tubers stored at 5 than those at 10^{o}C due to the sugar content differences.

Metabolite profiling has also been applied on potato suberization. Matsuda (2003) reported a new LC-MS method for the determination of the metabolic flux of two hydroxycinnamic acid conjugates, (S)-N-pcoumaroyloctopamine and chrologenic acid contained in wound-healing potato tuber tissue. Later, a GC-MS based metabolite profiling study on suberin biosynthesis was carried out by Yang (2007) using potatoes as wound-healing model. More recent publications on potato metabolomics refer to the potato metabolome changes affected by different agricultural production systems and the regulation

of metabolome by polyphenol oxidase in genetically modified potato tubers using LC- and GC/MS by Shepherd *et al.* in 2013 and 2014 respectively. In addition, the effect of storage upon polar and non-polar compounds in six potato cultivars was examined by Uri *et al.* (2014).

2.6. Conclusions

In conclusion, it is clear that there is a complete dearth of research which has investigated blackheart in potato; much of the literature is between 40 and 90 years old. Although some of the information is still relevant there is still no true understanding of the causal factors that govern blackheart susceptibility. More importantly, there is no understanding of the physiological, biochemical and indeed genetic mechanisms underlying resistance/susceptibility. Identification of predictive biomarkers of blackheart and elucidation of the role and effect that respiration and gaseous exchange have will ultimately enable storage practitioners and packers to reduce incidence and severity. If the mechanisms behind blackheart can be better understood, then specific consignments which are believed to be at risk (e.g. by having higher levels of predictive pre-symptomatic biomarkers) may be released in advance of blackheart becoming a problem later in storage.

CHAPTER THREE:
MATERIALS AND METHODS

3.1. Overview of work

Physiological and biochemical analysis of potato (*Solanum tuberosum* L.) stocks cv. Maris Piper that had different susceptibility to blackheart (BH) was performed in order to identify factors involved in the development of this physiological disorder. The potato stocks for all experiments were selected and transported from Sutton Bridge Crop Storage Research (SBCSR, UK) to Cranfield University (year 1 and 2) or to K.U. Leuven (Leuven, Belgium) (year 3). During year 1 (2011-2012) and year 2 (2012-2013), tuber respiration rate was examined in order to interpret the level of CO_2 produced when tubers were stored under different storage conditions (air or CA). Tuber weight and size (length and maximum equatorial diameter) were also recorded. O_2 diffusion in tuber tissues was measured in year 3 (2013-2014). Regarding the biochemical analysis, non-structural carbohydrates (sugars) and phenolic analysis were carried out in order to identify a possible link between these targeted compounds and tuber tissue discoloration. Untargeted metabolomics analysis was also applied with the possibility of discovering differences in those metabolites identified in flesh and heart tissues and tissue discoloration. Potato stock susceptibility in BH was also assessed using untargeted metabolomics analysis which was applied on control (sound samples showing no discoloration) samples derived from one susceptible and one non-susceptible stock. Biochemical analysis was not carried out for those samples of 2nd experiment in year 2 or in year 3.

In year 1 (2011-2012) three potato stocks with different susceptibility to BH were stored at 15^{o}C for shelf-life evaluation in air and/or CA on three sampling days. Those tubers were

initially stored at 1.5 or 3^{o}C and sampled at seven sampling points (outturns). For the whole experiment 1512 tubers were required in total. Respiration rate was measured in 1215 tubers in order the experiment to be balanced. That was because outturn 1 was conducted in air storage only and outturn 6 was conducted using tubers that were initially stored at 1.5°C only so this issue made the experiment to be unbalanced. 7560 samples in total including flesh, heart and peel tissues were cut, snap-frozen and stored pending further processing. From those samples only 648 (flesh and heart tissues) were selected from those tubers that have been stored in air only and initially stored at 1.5^{o}C and used for sugar and phenolic analysis. For untargeted metabolomics analysis 94 selected samples were used in total.

A similar pattern to year 1, for year 2 (2012-2013) experiments was followed. Three Maris Piper potato stocks with different susceptibility to BH, two susceptible and one non-susceptible were used. Approximately 1000 potato tubers were initially stored at 1.5^{o}C until required. For the 1st experiment 324 tubers were required in total. The experiment (from 7/12/2012 to 10/05/2013) was slightly different compared to that of year 1, where potato tubers were stored at 20^{o}C in air only, initially stored at 1.5^{o}C and sampled on two sampling days. All tubers were also used for respiration rate which was measured for each tuber separately. 1620 samples (flesh, heart and peel tissues) in total were snap-frozen and cut, yet only 96 were used for sugar and phenolic analysis. Untargeted metabolomics analysis was applied on 112 samples in total. For the 2nd experiment 459 potato tubers were used in total. Those tubers were initially stored at 1.5^{o}C for ca. 8 months. The purpose of this experiment, which started on the 19th June 2013 and lasted 14 days (ending on the 3rd July 2013), was to store tubers at 20^{o}C under various gas combinations (*viz*. A: 21% O_2, B: 10% CO_2, C: 10% O_2 and D: 5% O_2) in order to see whether different environmental conditions would influence the incidence of blackheart in potatoes. The respiration rate was recorded for each tuber separately.

In Year 3 (2013-2014) a gas diffusivity experiment was conducted from the 30th of May 2014 until 13th of June 2014 at the Faculty of Bio-science Engineering (K.U.Leuven) in Leuven, Belgium. The idea of this experiment was to use two potato stocks cv. Maris Piper (one susceptible and one non-susceptible) and to record the O_2 levels in potato tuber tissues (flesh and heart) in order to find any possible relation with the blackheart disorder induction. 40 potato tubers (20 tubers / stock) were needed for the whole experiment.

3.2. Plant material and experimental design

3.2.1. Year 1 (2011-2012)

A method was developed at Sutton Bridge Crop Storage Research (SBCSR, UK) for BH induction in order susceptible and non-susceptible to BH potato tuber stocks to be selected and transferred at Cranfield University (UK). Fifteen stocks of potato (*Solanum tuberosum* L.) cv. Maris Piper (supplied by FPSA member companies) were initially stored at 3.5°C and sealed in chambers at 30°C for 60 h incubation. Thus, potato stocks were adjudged by SBCSR to be either susceptible or non-susceptible to BH. Three stocks of potato tubers of which stock 20 and stock 23 were considered particularly susceptible to BH (Fig. 3.1 a, b) and stock 12 was proposed as non-susceptible to BH (Fig. 3.1c) were collected from SBCSR and transported to Cranfield University on the 24th of November 2011 within two hours. Baseline and six sampling points (outturns) were conducted at Plant Science Laboratory (PSL) from November 2011 to May 2012.

According to the experimental design (Fig. 3.2) two different initial storage temperatures were selected (1.5 and 3°C). The warmer temperature was considered as a typical storage temperature for pre-pack industry whilst the storage temperature of 1.5°C was selected as being more challenging for the tubers. The duration of the experiment was 24 weeks (*viz.* 4, 8,

12, 16, 20 and 24 weeks). Assessments were made on one baseline and six sampling point (outturns). Tubers were sampled at three different sampling days (*viz.* 0, 3 and 7 days) per four weeks (outturn) at 15^{o}C for shelf-life evaluation, whilst the rest of the stocks remained stored at 1.5 and 3^{o}C until required. On each sampling day 0 tubers were always stored in air (21% O_2) and on each sampling days 3 and 7 storage took place in air or controlled atmosphere (CA) (18-19% O_2 and 10% CO_2) (Fig. 3.2). Storage at 15^{o}C in air and/or CA was conducted in six (6) sealed boxes 300 L each (3 with air conditioning and 3 with CO_2 conditioning) using a CA system (Fig. 3.6) where sampling replicates of each stock were divided, respectively. Baseline was conducted without initial storage temperature as the tubers were sampled directly after their arrival at Cranfield University. The arrival day was considered as sampling day 0, whilst the rest of the tubers required for the baseline measurements were directly stored at 15^{o}C in air or CA in order to be processed on sampling days 3 and 7. Using a pocket thermometer (Fig. 3.1d) three hours were required for conditioning when tubers transferred from 1.5 or 3^{o}C to 15^{o}C (temperature equilibrium establishment). For the whole experiment 1512 tubers were required (504 tubers / stock).

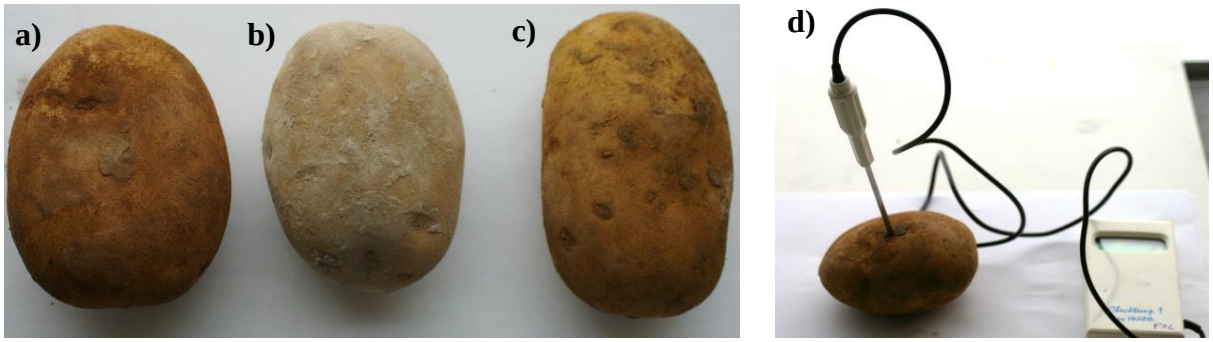


Figure 3.1 Potato tubers cv. Maris Piper stock 20 (a), stock 23 (b) (both susceptible to BH) and stock 12 (non-susceptible to BH) (c). Pocket thermometer (d).

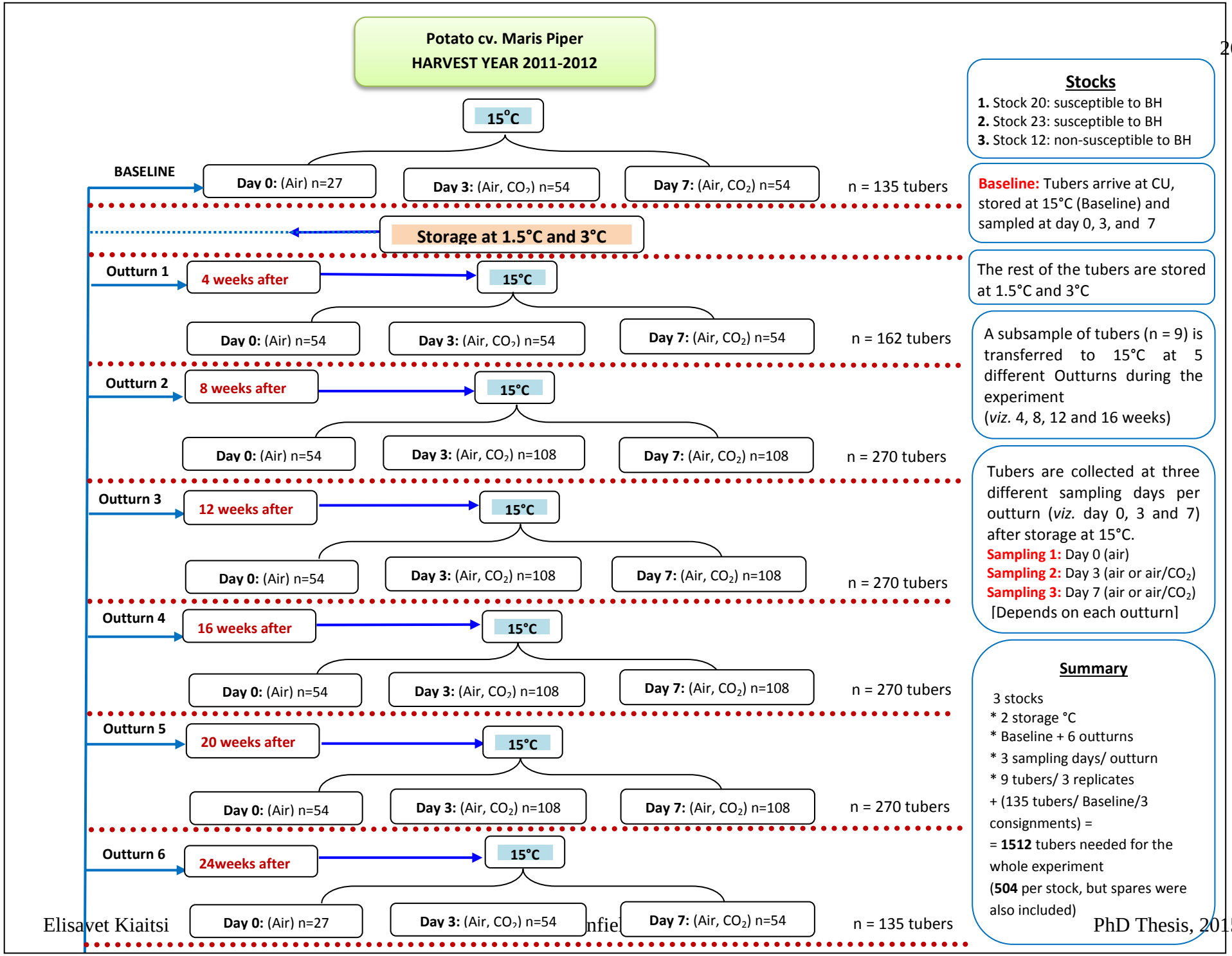


Figure 3.2 Experimental design year 1 (2011-2012)

3.2.2. Year 2 (2012-2013)

3.2.2.1. Experiment 1

Three stocks of potato tubers cv. Maris Piper [stock 7 (susceptible to BH) (Fig. 3.4a), stock 12 (susceptible to BH) (Fig. 3.4b) and stock 3 (non-susceptible to BH) (Fig. 3.4c)] were transported from SBCSR to Cranfield University on the 7th of December 2012 within four hours. Tuber stocks were adjudged by SBCSR to be either susceptible (stock 7 and stock 12) or non-susceptible (stock 3) to BH. According to the experimental design (Fig. 3.3) assessments were made on a baseline and five sampling points (outturns) where each outturn was conducted every four weeks. This experiment was conducted at Plant Science Laboratory (PSL) from December 2012 to May 2013 and lasted 20 weeks (*viz.* 4, 8, 12, 16 and 20 weeks). Tubers were sampled at two different sampling days per outturn (*viz.* 0 and 7 days) in air (21% O_2) only at 20^{o}C for shelf-life evaluation. No initial storage temperature took place during baseline measurement as the arrival day of the tubers was considered as sampling day 0 and tuber subsamples (n= 9 tubers / stock) were immediately sampled. Another subsample of 27 tubers was transferred directly to 20^{o}C in air to be processed on sampling day 7. For the baseline measurement 54 tubers were used in total. The rest of the stocks remained stored at 1.5^{o}C pending further processing. The tubers had to be warmed up for five to six hours when transferred from 1.5 to 20^{o}C (temperature equilibrium establishment). A pocket thermometer was used to control the temperature equilibrium (Fig. 3.4d). For the whole experiment 324 tubers were required in total (108 tubers / stock) (Fig. 3.3).

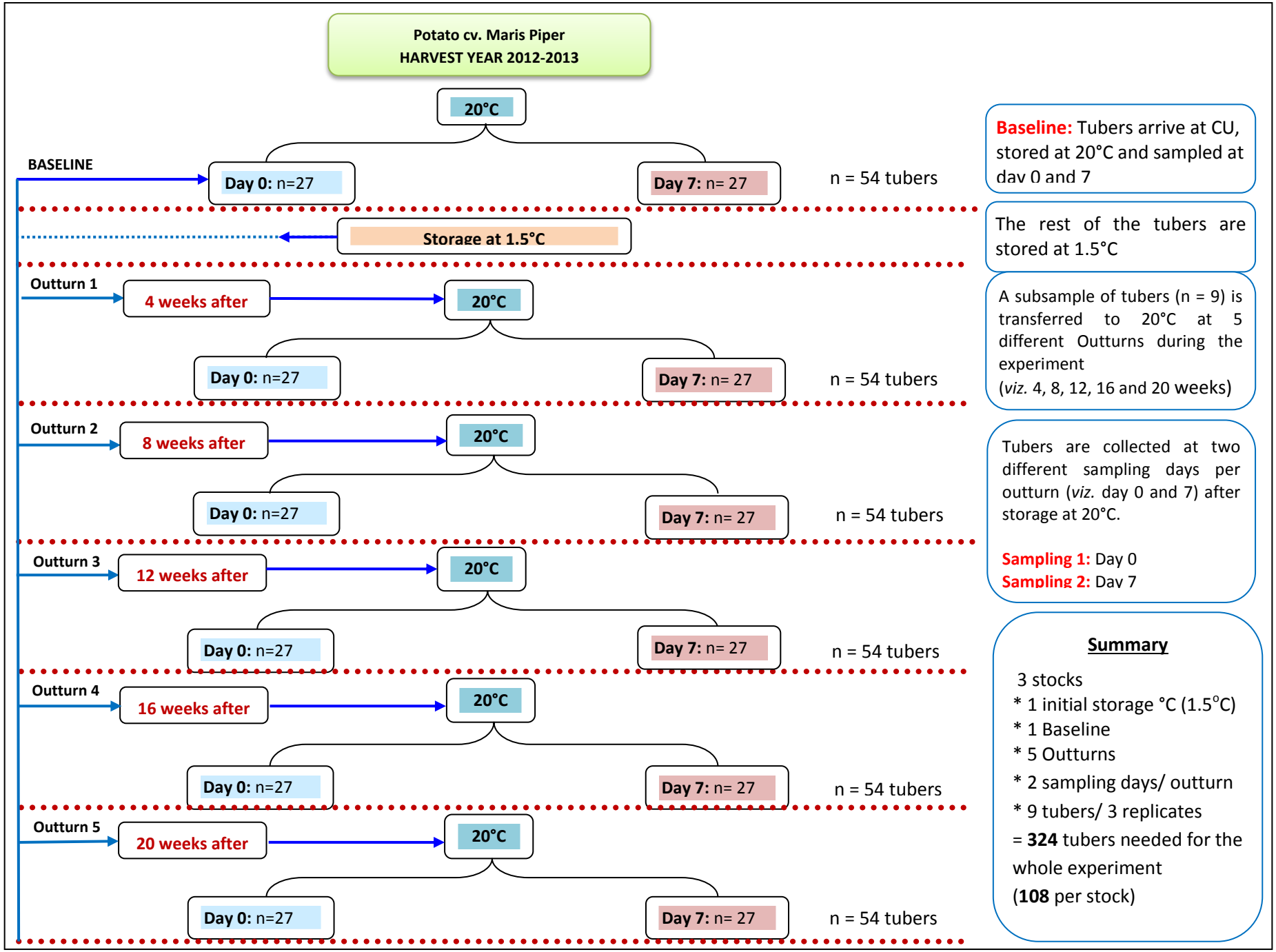


Figure 3.3 Experimental design Year 2 – Experiment 1 (2012-2013)

3.2.2.2. Experiment 2

As previously mentioned in *section 3.2.2.1.*, three stocks of potato tubers (*Solanum tuberosum* L. cv. Maris Piper) [stock 7 (susceptible to BH), stock 12 (susceptible to BH) and stock 3 (non-susceptible to BH)] (Fig. 3.4) were transported from SBCSR to Cranfield University. Potato tuber stocks were stored at cold storage (1.5^{o}C) for ca. 8 months. According to the experimental design (Fig. 3.5), assessments were made on five sampling days [*viz*. 0 (baseline), 3, 7, 10 and 14 days]. Baseline (sampling day 0) was carried out using 27 tubers (9 tubers / stock) that were placed on plastic trays, stored in air, equilibrated at 20^{o}C and sampled, whilst the rest of the tubers (n= 432) were stored in various gas combinations (*viz*. A: 21% O_2, B: 10% CO_2, C: 10% O_2 and D: 5% O_2) in order to be sampled on days 3, 7, 10 and 14 at 20^{o}C. Using a pocket thermometer five to six hours were required for the tubers to warm up when transferred from 1.5 to 20^{o}C (temperature equilibrium establishment). Storage under various gas combinations took place in 12 sealed boxes (300 L each box) using the CA system (Fig. 3.6). Three (3) boxes were used as replicates per gas combination. For the whole experiment 459 tubers were required (153 tubers / stock) (Fig. 3.5).

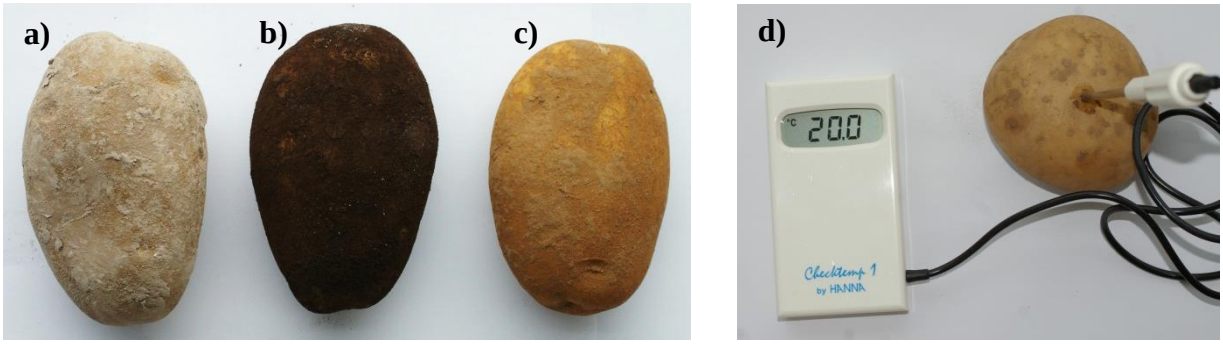


Figure 3.4 Potato tubers cv. Maris Piper stock 7 (a), stock 12 (b) (both susceptible to BH) and stock 3 (non-susceptible to BH) (c). Pocket thermometer (d).

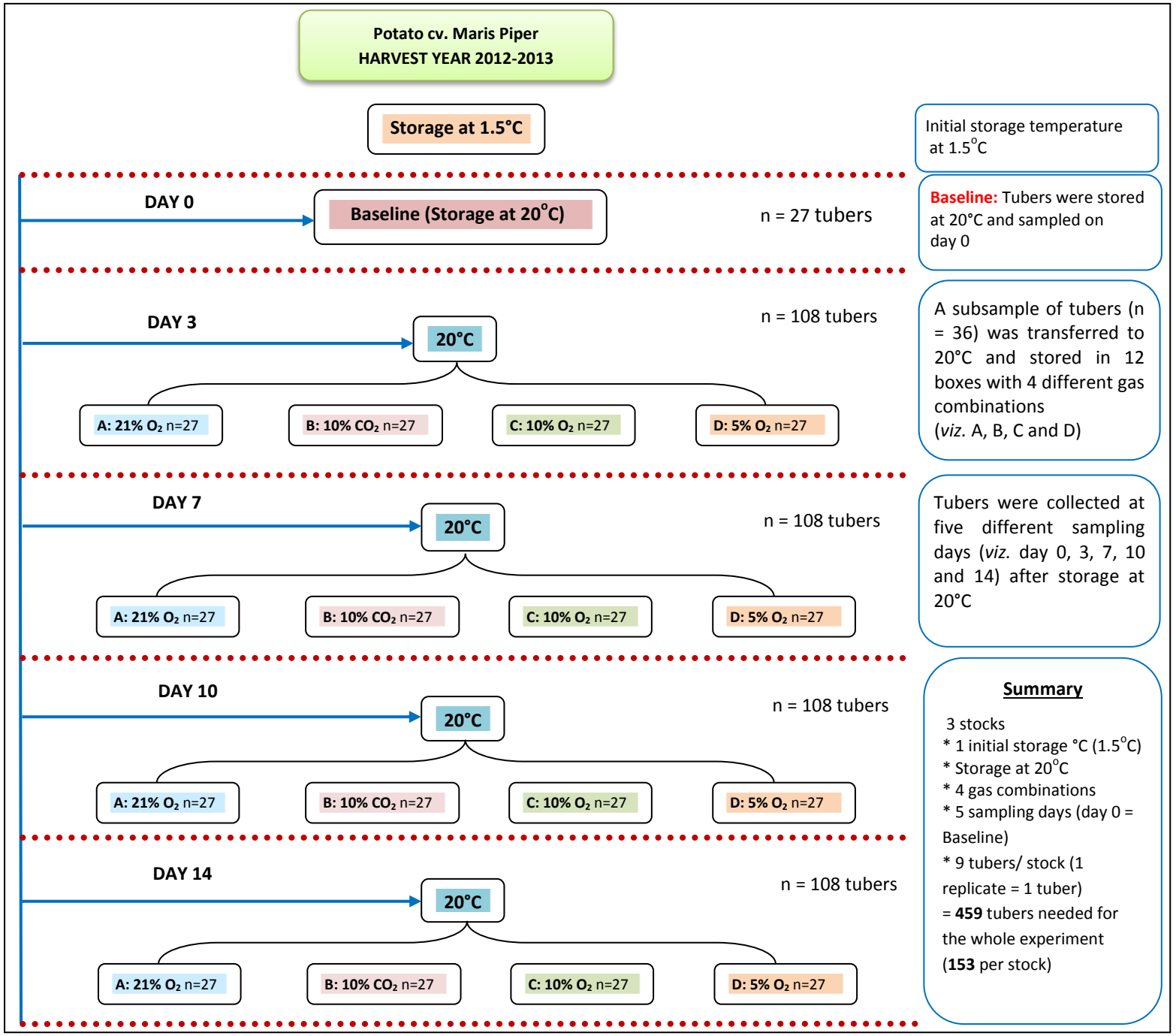


Figure 3.5 Experimental design Year 2 – Experiment 2 (2012-2013)

3.3. Shelf-life evaluation

Shelf life evaluation was carried out by storing potato tubers in air or controlled atmosphere (CA) using the ICA6000 system (International Controlled Atmosphere Ltd., Paddock Wood, Kent, UK) (Fig. 3.6a). The ICA6000 system (an automated sample sequencing system) was able to measure and control the gas concentration in storage boxes. Each box (300 L) was connected to the CA system with three tubes (sample in- and out-let, gas in-let) and a small fan was fixed in each box to circulate the gases in the boxes. This system was equipped with a carbon dioxide sensor where CO_2 could be measured over the range of 0 to 20% using an Infra-Red sensor and an oxygen sensor that the O_2 was measured over the range of 0 to 25% O_2 using an electrochemical measuring cell.

CA storage in year 1 (2011-2012) was conducted at 15^{o}C in six sealed boxes (Fig. 3.6b). Three boxes were used for air atmosphere where O_2 was measured approximately to 21% and CO_2 below 0.04% and the other three boxes were used for CA with a CO_2 concentration of about 10% in combination with O_2 between 18-19%. For the 2nd experiment in year 2 (2012-2013) potato tubers were stored at 20^{o}C in four (4) different gas combinations (*viz*. A: 21% O_2, B: 10% CO_2, C: 10% O_2 and D: 5% O_2) and CA storage took place in 12 sealed boxes (Fig. 3.6b). Three (3) boxes were used as replicates per gas combination.

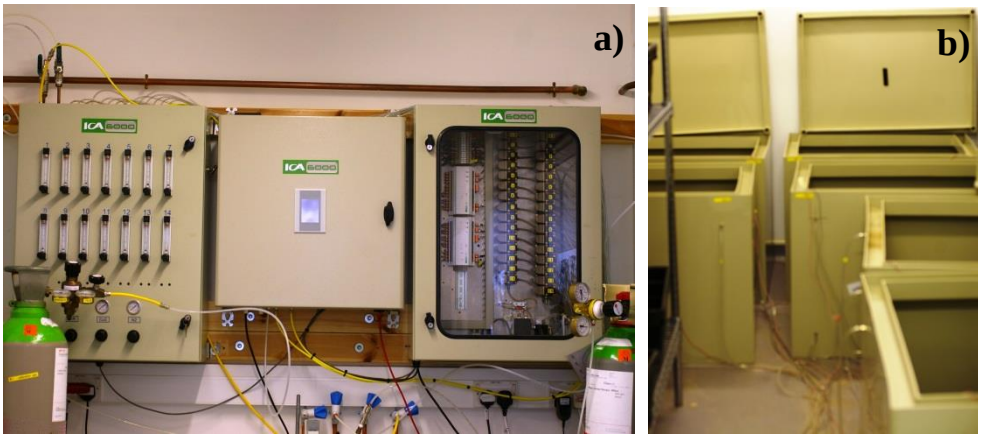


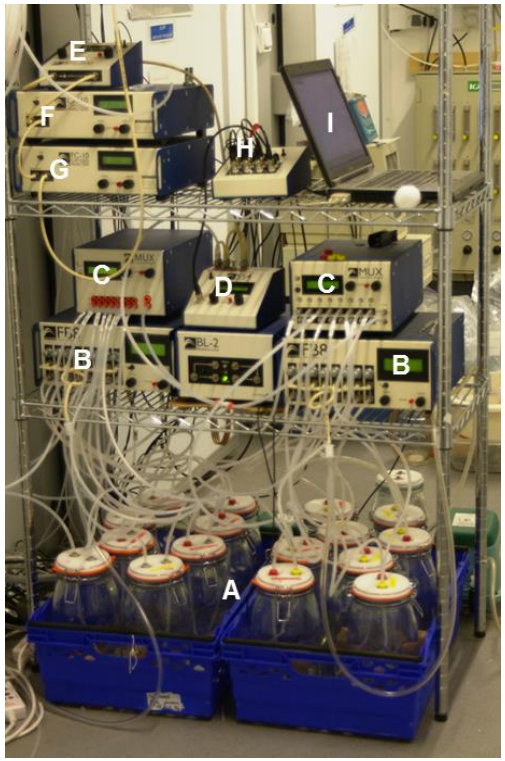
Figure 3.6 ICA6000 system (a), boxes (300 L each) (b).

3.4. Real time respiration rate

Using a Sable Respirometry System (Sable Systems, NV, USA) (Fig. 3.7) which was configured as a push mode respirometry on a dynamic system, potato tuber respiration rate measurements were carried out. The Sable Respirometry System was equipped with Gas Analyzers [(FC-10 Oxygen Analyzer (Fig. 3.7G), CA-10 Carbon Dioxide Analyzer (Fig. 3.7F) and RH-300 Water Vapour Analyzer (Fig. 3.7E)] and Gas Flow Switchers [(MUX Flow Multiplexer (Fig. 3.7B) and FB8 Flow Measurement System (Fig. 3.7C)] and it was calibrated using 10.06% CO_2 and 1.99% O_2 (10% CO_2, 2% O_2, 88% N_2; certified standard from BOC, Surrey, UK). Initially, sampled air was analysed by a RH-300 water vapour detector for water vapour pressure (WVP) determination and then passed through a water scrubber (Drierite). Subsequently, carbon dioxide and oxygen of the sampled air were analysed by a CA-10 carbon dioxide detector and a FC-10 oxygen detector respectively. Flow rate (FR, mL/min) and barometric pressure (BP, kPa) were recorded as well. The UI-2 universal interface (Fig. 3.7H) was connected to the subsampler (Fig. 3.7D), multiplexer and detectors, allowing the recording data to be interpreted and analysed by the computer (Fig. 3.7I) using the ExpeData software.

Potato tubers were placed in eight sealed jars (1 jar= 3 L) (Fig. 3.7A) one of which was dedicated as the baseline measurement (empty jar without tuber). Jars were ventilated with a known air flow (1 L/min) pushed throughout the system (use of gas flow switchers). Baseline measurements were carried out in order to prevent cross contamination occurring between samples and allowed for the initial CO_2 and O_2 levels to be accurately determined. The duration of the baseline measurement was recorded over 2 minutes. After, the subsampled air from each jar was analysed over a 2 minutes period, three times (cycles-repetitions), to provide one average reading. The mean CO_2 value was determined for the first baseline

measurement recorded during each analysis to allow normal atmospheric CO_2 concentrations to be removed from the respiration rate calculations. The Sable System was able to calculate the levels of CO_2 in ml h^{-1} based on the subsampling flow rate. Finally values were then adjusted for potato tuber weight (kg) to determine the respiration rate in ml CO_2 kg^{-1} h^{-1} for each jar. In year 1 (2011-2012) respiration rate was recorded by placing 3 tubers per jar. However, in both experiments in year 2 (2012-2013) only one tuber was placed in each jar separately.



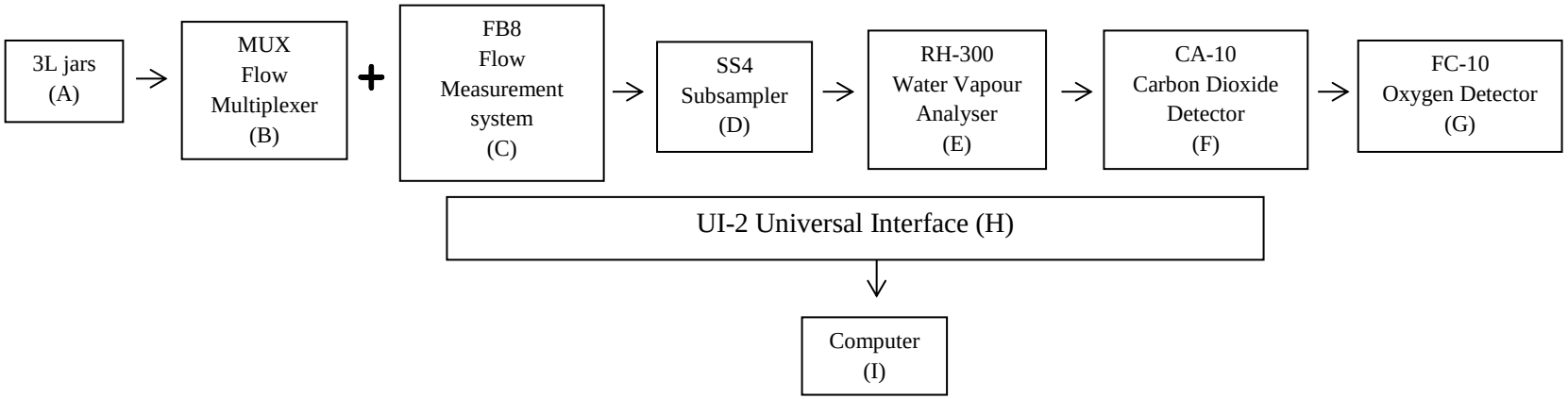


Figure 3.7 Picture and schematic diagram of Sable Respiratory System set up

3.5. Sample preparation

On removal from storage, potato tubers were carefully washed with tap water, wiped using roll paper and further left to air dry. Tubers were weighed using a balance, labelled and placed on plastic trays according to the sampling replicates of each stock (9 tubers / tray). Tuber size was also recorded and determined by measuring the length (mm) and the maximum equatorial diameter (mm) using a digital calliper. Then, real time respiration rate measurement was carried out using the Sable Respirometry system (Fig. 3.7). After this procedure, one tuber slice (10 mm in thickness) was cut longitudinally with a sharp knife from the central part of each tuber (Fig. 3.8a). The thickness of the sample was measured with a digital calliper. Using a cork borer a cylinder disc (24 mm in diameter) from the central part of the slice (heart; Fig. 3.8a) was cut and then divided in two half equatorial semi circles. The rest of the tuber slice was divided into flesh and peel (Fig. 3.8b). All tuber sections were immediately snap frozen in liquid nitrogen and one half of heart and flesh samples were stored at -80^{o}C (stock samples) whilst the other halves (second half heart and flesh) and whole peel were stored at -40^{o}C pending further processing (Fig. 3.8b). Fresh weight (FW) was recorded. Samples stored at -40^{o}C were freeze-dried in the dark (lyophilisation) using a digital freezing-drier (Scanvac, Lynge, Denmark) at -50^{o}C for seven days. After lyophilisation, dry weights (DW) of the samples were also recorded and then samples were stored back at -40^{o}C until required for biochemical assessments.

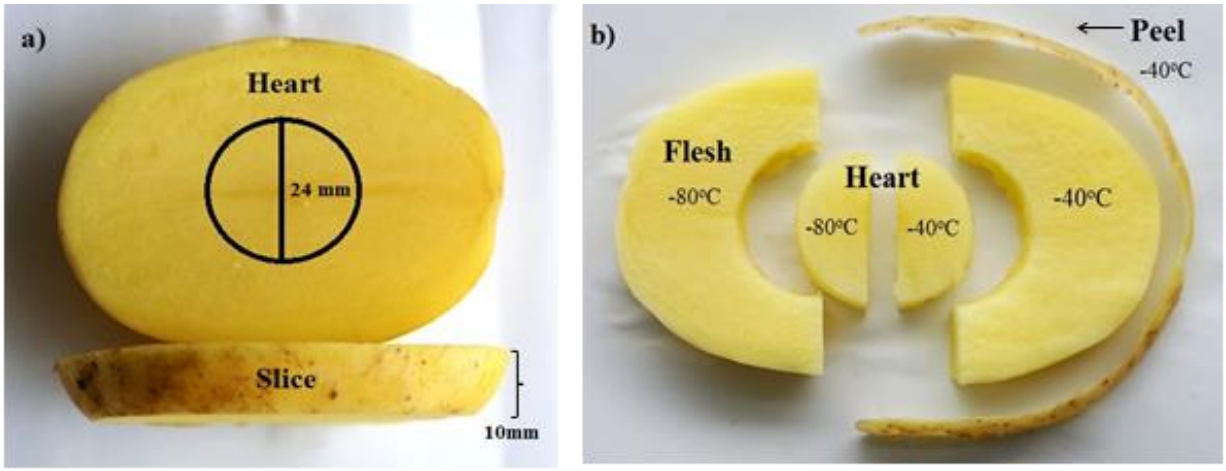


Figure 3.8 Tuber slice (10 mm in thickness) and heart (24 mm in diameter) (a), flesh, heart and peel samples before snap-freezing procedure (b).

3.6. Biochemical assessments

Biochemical assessments such as sugar analysis, phenolic analysis and untargeted metabolomics analysis were carried out in both years 1 and 2. In year 1 (2011-2012) sample selection for sugar and phenolic analysis was carried out from potato tubers cv. Maris Piper [Stock 20 (susceptible to BH), stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH)] (Fig. 3.1a, b and c, respectively) that had been stored in air only at 15^{o}C on three sampling days (*viz.* 0, 3 and 7 days). Potato tubers were initially stored in air at 1.5^{o}C for 8, 16 and 20 weeks. Baseline samples were also included. Sample selection was based on greater BH incidence observed in those tubers initially stored at 1.5^{o}C. Tissue discoloration was classified in four terms. The first one was called brown centre light (BCL); a light brown discoloration found in the heart part of the tuber, the second one brown centre (BC); a more distinguished brown discoloration in the very centre of the tuber, the third one pith; a light brown discoloration formed along the pith tuber area and the last one was named dark brown to black (BH); a dark brown to black more intense discoloration presented mainly in the centre tuber area. Figure 3.9 shows example of tuber samples of stock 23 (susceptible to BH) indicating tissue discolorations [*viz.* brown centre light (BCL) (Fig. 3.9a), pith (Fig. 3.9b),

brown centre (BC) (Fig. 3.9c) and dark brown to black (BH) (Fig. 3.9d)]. Further example figures of discoloured samples of stock 20 and stock 23 are present in Appendix A. Control samples (sound tubers showing no discolorations) were also included (Fig. 3.9e). 648 samples in total were used. Untargeted metabolomics analysis was conducted using selected samples of stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) (see section 3.6.3.).

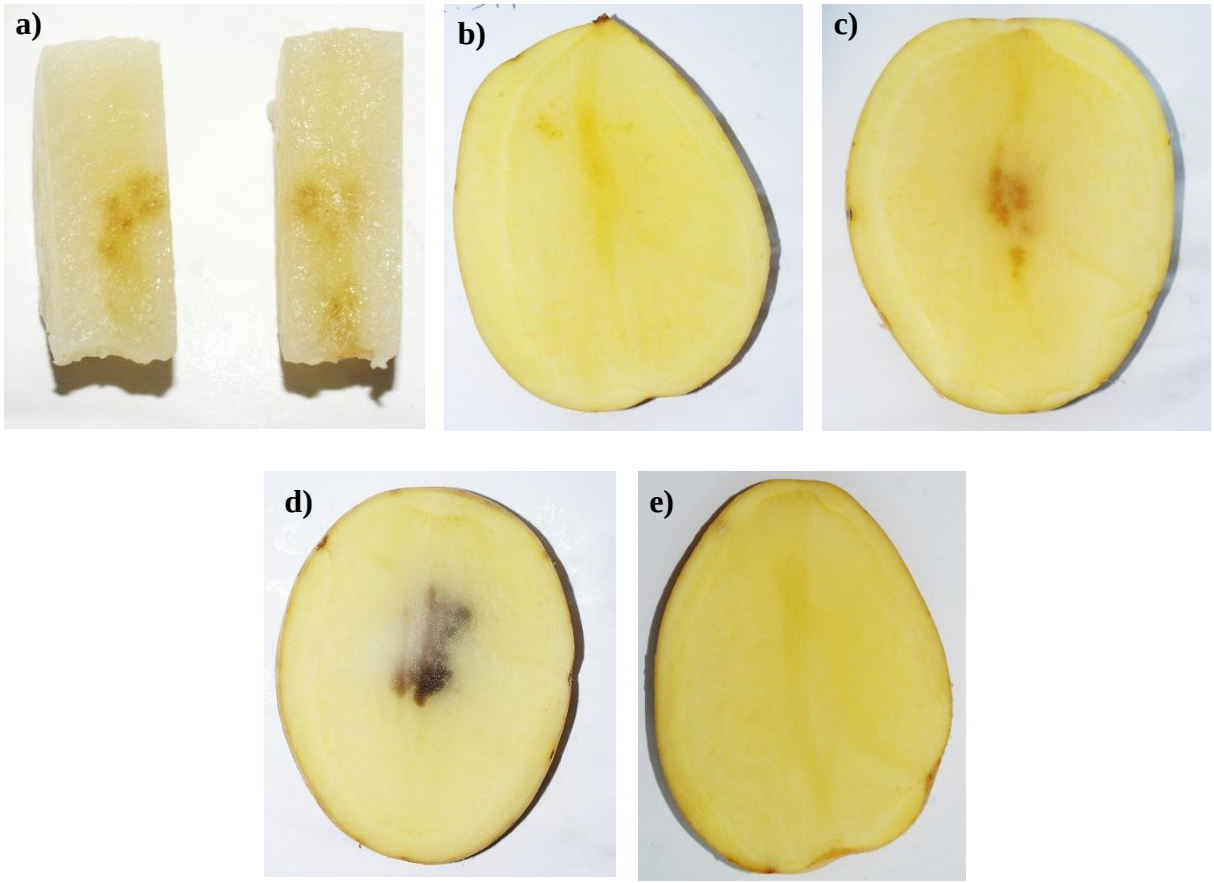


Figure 3.9 Examples of affected potato tubers cv. Maris Piper indicating tuber tissue discoloration as brown centre light (BCL) (a), pith (b), brown centre (BC), dark brown to black (BH) (d). Control tuber showing no discoloration (e).

In year 2 (2012-2013), biochemical analysis was only conducted on those samples of the 1st experiment and only two potato stocks were used [stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) (Fig. 3.3a and 3.3c, respectively). Tubers were stored in air

only at 20°C on two sampling days 0 and 7 and initially stored at 1.5°C for 4, 8, 12, 16 and 20 weeks. Baseline samples were also included. Stock 7 showed pith, brown centre light (BCL) and brown centre (BC) tissue discolorations (further info in Appendix B). Examples of affected potato tubers are pictured in Figure 3.10. Control samples were also included. Sugar and phenolic analysis was conducted on 96 samples in total that further used for untargeted metabolomics analysis (see section 3.6.3.).

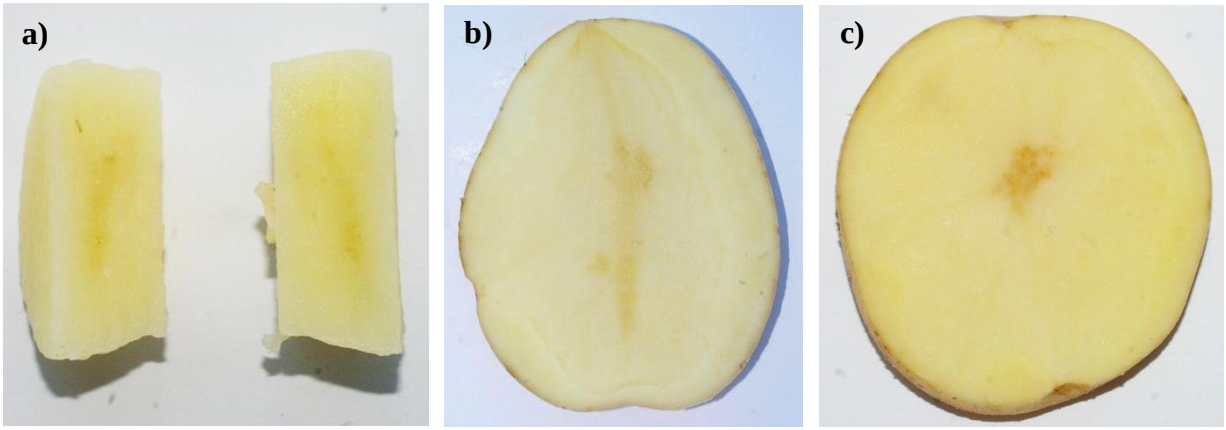


Figure 3.10 Examples of affected potato tubers cv. Maris Piper indicating tuber tissue discoloration as brown centre light (BCL) (a), pith (b) and brown centre (BC).

3.6.1. Extraction and quantification of non-structural carbohydrates

3.6.1.1. Sample extraction

Freeze-dried flesh and heart tuber tissue samples (50 mg) (Fig. 3.14a) were powdered using a grinder (Restch Gmbh, Haan, Germany), weighed in 1.5 ml microtubes (Eppendorf type polypropylene microtubes, Deltalab, Rubi, Barcelona, Spain) and extracted with 0.75 ml of 62.5:37.5 HPLC grade methanol:water (v/v). The microtubes were placed in a shaking water bath at 55°C for 15 min and every 5 min they were removed and vortexted (Vortex Genie 2, Scientific Industries, NY) at room temperature for 20 sec. After cooling, the samples were centrifuged for 10 min at 10,000 rpm (rotations per minute). Subsequently, the

supernatant was filtered through a 0.2 μm filter (Cronus PTFE filters, Jaytee Biosciences Ltd., Kent, UK) driven by a 3 ml syringe (Fig. 3.14c) and stored at -40^{o}C until further analysis.

3.6.1.2. HPLC-ELSD parameters

Sugar (fructose, glucose and sucrose) analysis was conducted using a High Performance Liquid Chromatography Agilent 1260 series coupled to Infinity Evaporative Light Scattering Detector (ELSD) (Cheshire, UK) (Fig. 3.11). Initially, the extracts were diluted (1:4). Year 1 (2011-2012) and year 2 / 1st experiment (2012-2013) samples (20 μl) were injected into a Prevail Carbohydrate ES 5u (GRACE) 250 mm x 4.6 mm column at a flow of 1.0 mL/min and the column temperature was set at 30^{o}C. Gradient elution was performed with a mobile phase HPLC grade water (solution A) and HPLC grade acetonitrile (solution B) as follows: time 0-15 min, 20% A, 80% B; 15-20 min, 50% B, 50% A; 32% B; 20 min, 20% A, 80% B. Run time per sample was 20 min. Sugar concentrations were calculated against authentic calibration standards of fructose, glucose and sucrose ranging from 0.1 to 5 mg mL^{-1} (Sigma, Dorset, UK) (Fig. 3.12).

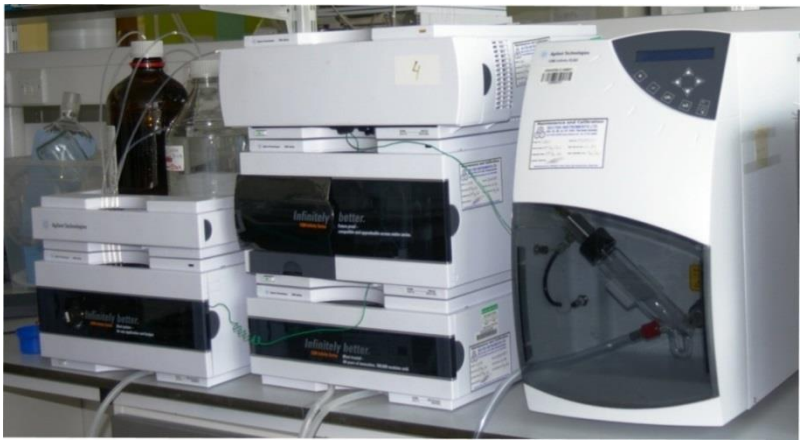


Figure 3.11 High Performance Liquid Chromatography (HPLC) Agilent 1260 series coupled to Infinity Evaporative Light Scattering Detector (ELSD) instrument.

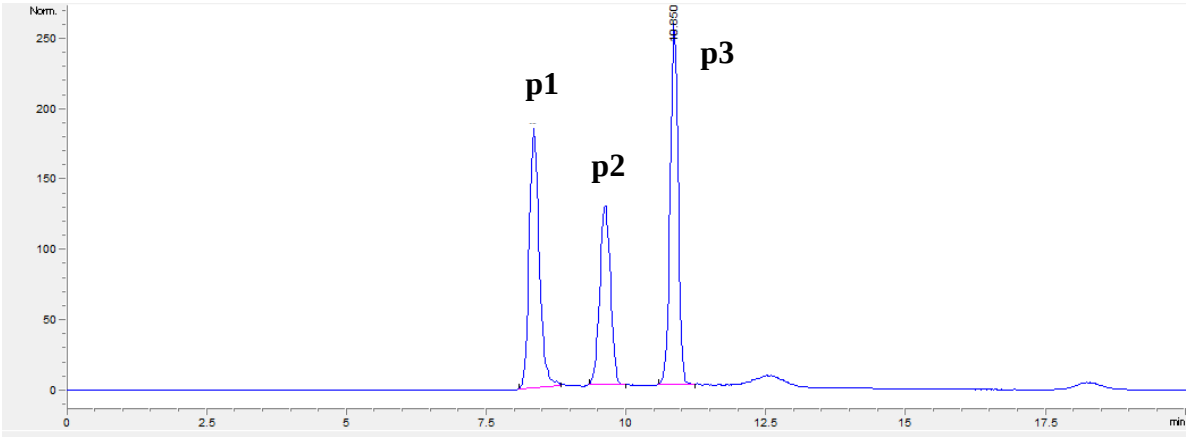


Figure 3.12 Example of standard chromatogram with elution order: Fructose (peak 1), glucose (peak 2) and sucrose (peak 3).

3.6.2. Extraction and quantification of phenolic compounds

Targeted analysis of phenolic compounds in potato tubers cv. Maris Piper stocks from year 1 and 2 was conducted using a Liquid Chromatography (LC) Agilent Technology 1290 Infinity coupled with Agilent Technologies 6540 GHD Accurate-Mass Quadrupole Time of Flight (Q-ToF) mass spectrometer (MS) instrument (Cheshire, UK) (Fig. 3.13). Individual phenolic compounds such as hydroxycinnamic acids and derivatives (chlorogenic, neo-chlorogenic, crypto-chlorogenic, caffeic, ferulic, *p*-coumaric and *p*-coumaroylquinic acid), caffeoyl-D-glucose, flavonols [rutin, quercetin-3,4-O-diglucoside (Q-3,4-O-di), isorhamneti-3-rutinoside and isorhmanetin-3-glucoside], hydroxycinnamic acid amides (caffeoylputrescine and ferulyolputrescine) and aromatic amino acids (tyrosine, phenylalanine and tryptophan) were detected and quantified in flesh and heart tuber tissues.

Figure 3.13 Liquid Chromatography (LC) coupled with Accurate-Mass Quadrupole Time of Flight (Q-ToF) mass spectrometer (MS) instrument.

3.6.2.1. Sample extraction

Freeze-dried flesh and heart tuber tissue (50 mg) samples (Fig. 3.14a) were powdered using a grinder (Restch Gmbh, Haan, Germany), weighed in 2 ml microtubes (Eppendorf type polypropylene microtubes (Deltalab, Rubi, Barcelona, Spain) and extracted with 1.5 ml of 50:50 HPLC grade methanol:water (v/v) + 1% formic acid (HCOOH) (Fig. 3.14b). The microtubes were placed in a shaking water bath at 35^{o}C for 15 min and every 5 min they were removed and vortexted (Vortex Genie 2, Scientific Industries, NY) at room temperature for ca. 20 sec. After cooling, the samples were centrifuged for 10 min at 10,000 rpm (rotations per minute). Subsequently, the supernatant was filtered through a 0.2 μm filter (Cronus PTFE filters, Jaytee Biosciences Ltd., Kent, UK) driven by a 3ml syringe (Fig. 3.14c) and stored at -40^{o}C until analysis. 648 and 96 samples from year 1 (2011-2012) and experiment 1 in year 2 (2012-2013) respectively were extracted.

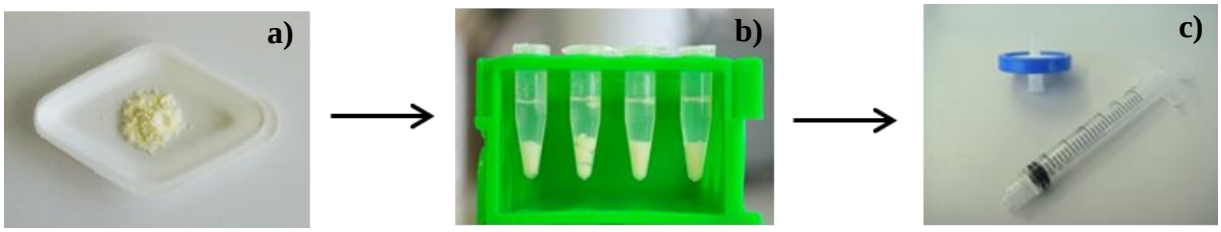


Figure 3.14 50 mg freeze dried powder (a) mixed with 1.5 mL of 50:50 (v/v) Methanol:water + 1% HCOOH (formic acid) (b) and filtered through 0.2 μm (c).

3.6.2.2. Liquid Chromatography/Mass Spectrometry (LC/MS) parameters

Phenolic analysis of potato tuber samples (10 μl injection) was conducted using an ElectroSpray Ionisation (ESI) source in negative mode on an Agilent Technology 1290 Infinity UPLC coupled with Agilent Technologies 6540 GHD Accurate-Mass Quadrupole Time of Flight (Q-ToF) mass spectrometer (Fig. 3.13). Chromatography was performed on a WATERS – ACQUITY UPLC C18 2.1 x 150 mm 1.7 Micron column (WATERS, Ireland, UK) with a gradient of eluent A: 0.1% (v/v) formic acid for LC/MS in HPLC grade water and eluent B: acetonitrile for LC/MS + 0.05% formic acid for LC/MS. Flow rate was set at 0.4 ml/min. The mobile phase was as follows: time 0 min, 95% A, 5% B; 0.5 min, 95% A, 5% B; 2.5 min, 81% A, 19% B; 6 min, 81% A, 19% B; 15 min, 60% A, 40% B; 15.50 min, 60% A, 40% B; 15.6 min 100% B; 17.6 min; 100% B; 17.65 min, 95% A, 5% B; 20 min. 95% A, 5% B. Run time per sample was 21 min.

3.6.2.3. Quantification

Quantification of phenolic compounds was carried out using chromatographic peaks that were identified according to their retention times compared against external standard compounds ranging from 20 to 10000 ng ml^{-1} and then concentrations of phenolic compounds were calculated in μg g^{-1} DW. Two standard calibration curves were used for better

separation of chlorogenic acid and crypto-chlorogenic due to their same molar mass [353.0878 $(M-H)^-$] and close retention time (Fig. 3.15). However, crypto-chlorogenic acid is eluted some seconds earlier than chlorogenic acid.

The first mix contained neo-chlorogenic acid (Fig. 3.15 p4), chlorogenic acid (Fig. 3.15 p6), caffeic acid (Fig. 3.15 p7), quercetin-3,4-*O*-diglucoside (Fig. 3.15 p8), p-coumaric acid (Fig. 3.15 p9), ferulic acid (Fig. 3.15p11), isorhamnetin-3-rutinoside (Fig. 3.15 p12) and isorhamnetin-3-glucoside (Fig. 3.15 p13). The second mix contained tyrosine (Fig. 3.15 p1), phenylalanine (Fig. 3.15 p2), tryptophan (Fig. 3.15 p3), crypto-chlorogenic acid (Fig. 3.15 p5) and rutin (Fig. 3.15 p10). Each external standard compound, except Tyrosine, was dissolved with 50:50 methanol:water (v/v). Tyrosine dialysis was conducted using 1 M HCl combined with heating. 500 mg of tyrosine powder were weighed in a beaker and then 5 ml of 1M HCl added in order to have a final concentration of 100 mg ml^{-1}. Heating at 50^oC followed for ca. 10 min until complete dialysis of tyrosine.

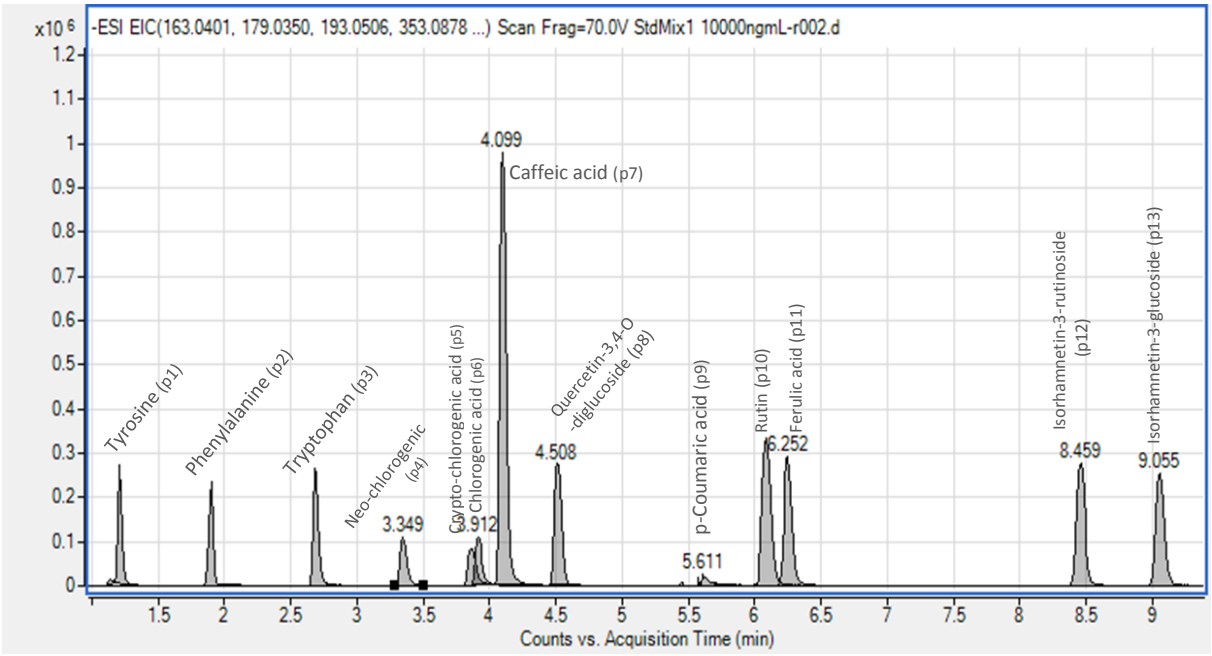


Figure 3.15 Example of LC/MS chromatogram of external standards (mix1 and 2 combined).

Furthermore, tandem mass spectrometry (MS/MS) was applied on a few samples (flesh and heart) derived from both first years (1 and 2) in order to identify five more phenolic compounds existed in potato tuber tissue and can be quantified with previous external standards used. Caffeoylputrescine, ferulyolputrescine (hydroxycinnamic acid amides) and p-coumaroylquinic acid could not be detected in those samples analysed. However, great abundance of caffeoyl-D-glucose and feruloylquinic acid was observed. Quantification of those compounds identified was carried out using linear equations (y= ax*b, R^2= 0.999) derived from calibration curves provided when external standards that have been previously used to quantify relevant compounds using the Agilent Quantitative Q-ToF software. Caffeoyl-D-glucose and feruloylquinic acid were quantified using corresponding linear equations of caffeic acid (Fig. 3.16a) and ferulic acid (Fig. 3.16b), respectively.

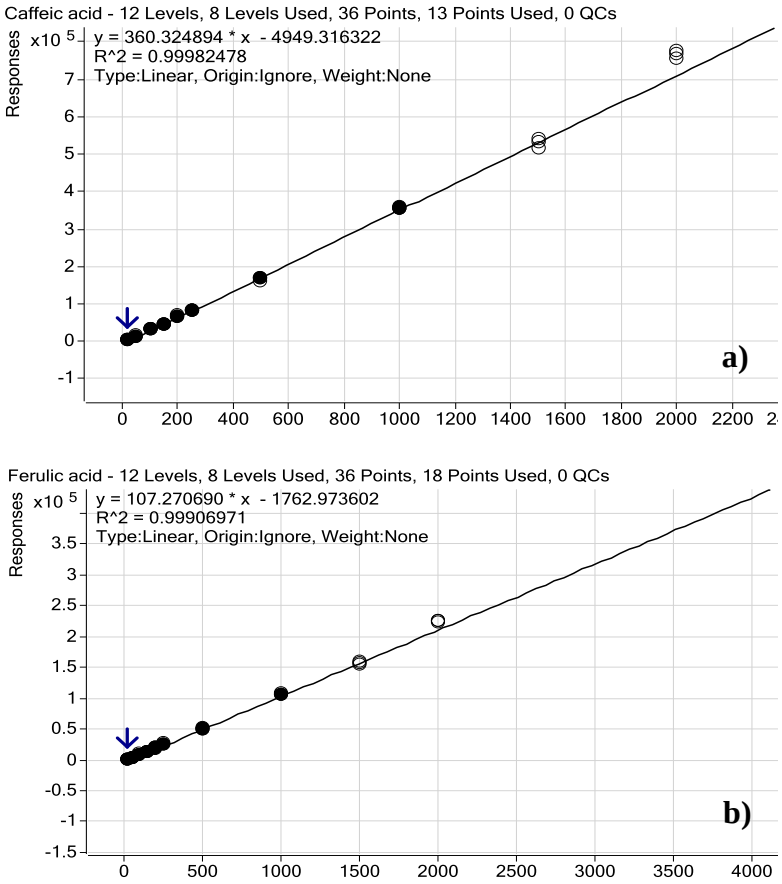


Figure 3.16 Examples of caffeic acid (a) and ferulic acid (b) calibration curves respectively. Linear equations are shown.

3.6.3. Untargeted metabolomic analysis

Untargeted metabolomic analysis was applied on potato tubers cv. Maris Piper derived from both year 1 and year 2 (experiment 1) in order to identify compounds which are possibly related to tissue discoloration and linked with blackheart development. The analysis was performed using a Liquid Chromatography (LC) Agilent Technology 1290 Infinity coupled with Agilent Technologies 6540 GHD Accurate-Mass Quadrupole Time of Flight (Q-ToF) mass spectrometer (MS) instrument (Fig. 3.13). Data were collected in positive and negative mode.

In both years, the analysis was separated in 2 sections.

The first section was carried out for metabolite identification between affected and non-affected tuber tissues (flesh and heart) derived from only one susceptible stock. Potato tubers from year 1 (2011-2012) that had different susceptibility to BH disorder were selected from tubers which were initially stored at 1.5^{o}C in air only for 12, 16 and 20 weeks of storage (outturn 3, 4 and 5 respectively). That was due to the highest BH incidence observed under those initial storage conditions. Tuber samples were chosen from stock 23 (susceptible to BH) which showed greater BH susceptibility during all outturns compared to stock 20 (susceptible to BH). Tuber tissue discolorations were classified as, brown centre light discoloration (BCL) (Fig. 3.9a), pith discoloration (Fig. 3.9b) brown centre discoloration (BC) (Fig. 3.9c) and dark brown to black discoloration (BH) (Fig. 3.9d). 12 affected tubers (3 tubers = 3 biological replicates/discoloration). Control samples (sound samples showing no discoloration) (Fig. 3.9e) were also included. Three non-affected tubers (as control) were selected from outturn 4 (week 16) on sampling day 7. A total of 30 samples were used in total (15 flesh and 15 heart tissues). Potato tuber selection for year 2 (experiment 1) was carried out from stock 7 (susceptible to BH). The tubers were initially stored at 1.5^{o}C for 4, 8, 12, 16

and 20 weeks of storage. In the 1st experiment, the tissue discoloration classification was carried out differently compared to year 1. That was due to the balance needed for statistical analysis later. So, tuber tissue samples (flesh and heart) were categorized as affected (A) and non-affected (control) samples. 64 samples were used in total (n=16 samples/tissue/discoloration).

The second section was conducted using control samples only derived from one susceptible against another one non-susceptible to BH stock in order to find metabolic differences in stock susceptibility. In terms of the parameter 'susceptibility' control samples that were derived from a susceptible stock were categorized as Y (yes = susceptible) and from a non-susceptible stock as N (no = non-susceptible). Year 1 control sample selection was carried out from tubers that have been stored in air only at 15^{o}C and initially stored at 1.5^{o}C for 8, 12, 16 and 20 weeks of storage (outturn 2, 3, 4 and 5 respectively). At each outturn control samples (flesh and heart) from the susceptible stock 23 (n= 12 flesh and 12 heart samples) were matched with those control ones from the non-susceptible stock 12 (n= 12 flesh and 12 heart samples). 48 samples in total were analysed. Control sample selection for year 2 was carried out from tubers of stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) that were stored in air at 20^{o}C and initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks of storage (outturn 1, 2, 3, 4 and 5 respectively) Baseline samples were also included. Similar with year 1, at each outturn control samples (flesh and heart) from both stocks were matched. 64 samples (16 flesh and 16 heart samples/stock/susceptibility) were analysed in total.

3.6.3.1. Data Filtering, Statistical Analysis and Visualization

The multivariate software Agilent Mass Profiler Professional 12.6 (MPP) was used for data filtering, visualization and advanced statistical significance analysis. Principal Component Analysis (PCA; unsupervised analysis), Analysis of Variance (ANOVA), and Fold Change analysis were used as statistical tools.

Initially, all chromatograms were extracted from LC/MS in both ion modes (negative and positive) exported as Extracted Compound Chromatogram (ECC) using the Agilent MassHunter Qualitative Analysis B.06.00 software and then converted as 'compound exchange formatted' (cef) files and eventually exported to the MPP software for statistical analysis and interpretation (see example chromatograms from years 1 and 2 in Appendix D; Figure 1.1-1.8 and Appendix E; Figure 1.1-1.2 respectively). At each year, four 'experiments' were created (n = 2 experiments / ion mode) (see Chapter 3: section 3.6). Independent variables and the attribute values of the independent variables must be specified to define grouping of the samples. Tuber tissue parameters (flesh and heart) and tissue discoloration [BC, brown centre; BCL, brown centre light; BH, dark brown to black; pith and control) parameters (non-numeric) were referred as 'conditions'. MPP software allows the creation of several interpretations between the 'conditions' with the option of pairing the parameters and single interpretation of one 'condition' only within an 'experiment'. In order to assess metabolomic differences between affected and non-affected tuber tissues, 'experiments A, B and E, F' of year 1 and year 2 respectively (discoloration only and tissue vs. discoloration interpretations) were created and seven interpretations were made in year 1 and two in year 2 (Table 6.1 and 6.2 respectively). In both years, assessment of metabolomic differences between control samples of potato stocks with different susceptibility to BH, two 'experiments' (C, D for year 1 and G, H for year 2) (susceptibility only and tissue vs.

susceptibility interpretations) were created and conducted using tuber tissue (flesh and heart control samples only) and stock susceptibility [Y = yes (susceptible stock) and N = no (non-susceptible stock)] as conditions (Table 6.3). In total, four experiments (n = 2 / ion mode) and 14 interpretations (n = 7 / ion mode) in total were conducted in year 1. Four experiments (n = 2 / ion mode) and 8 interpretations (n = 4 / ion mode) in total were carried out in year 2 (Table 6.3).

Table 3.1 Conditions and interpretations used in both 'experiments A (negative mode) and B (positive mode)' of year 1 samples of stock 23 (susceptible to BH) (BC, brown center; BCL, brown centre light; BH, dark brown to black discoloration).

Conditions		Interpretations	
Tuber tissue	Tissue discoloration	Discoloration only	Tissue vs. discoloration
FLESH (F)	BC	BC vs. control	FBC vs. FC HBC vs. HC
HEART (H)	BCL	BCL vs. control	FBCL vs. FC HBCL vs. HC
	BH	BH vs. control	FBH vs. FC HBH vs. HC
	pith	pith vs. control	Fpith vs. FC Hpith vs. HC
	control (C)		

Table 3.2 Conditions and interpretations used in 'experiments E (negative mode) and F (positive mode)' of year 2 [samples of stock 7 (susceptible to BH)].

Conditions		Interpretations	
Tuber tissue	Tissue discoloration	Discoloration only	Tissue vs. discoloration
FLESH (F) HEART (H)	Affected (A) control (C)	A vs. control	FA vs. FC HA vs. HC

Table 3.3 Conditions and interpretations used in 'experiment C (negative mode) and D (positive mode)' of year 1 and 'experiment G (negative mode) and H (positive mode)' of year 2 (control samples only).

	Conditions		Interpretations	
	Tuber tissue	Susceptibility	Susceptibility only	Tissue vs. Susceptibility
Year 1	FLESH (F) HEART (H)	Stock 23 (susceptible to BH); Y = yes susceptible / Stock 12 (non-susceptible to BH); N = no susceptible	Y vs. N	FN vs. FY HN vs. HY
Year 2	FLESH (F) HEART (H)	Stock 7 (susceptible to BH); Y = yes susceptible / Stock 3 (non-susceptible to BH); N = no susceptible	Y vs. N	FN vs. FY HN vs. HY

Many steps need to be followed in order to perform the analysis with the MPP software. At each interpretation of an 'experiment', quality control on samples was performed and all metabolites were initially filtered based on their frequency of presence in those samples interpreted where that filtering removed irreproducible metabolites. Filtering conditions included detainment of metabolites that appeared in at least 100% of samples in at least one condition. Then metabolites were additionally filtered based on a coefficient of variation of 10 or 25%.

After quality control on samples, statistical evaluation of the data was performed using two distinct statistical tools. Firstly, Principal Component Analysis (PCA) was conducted in order to summarize all those metabolites remained after quality control generating the components on a 3D scatter plot for visualization and the PCA scores were

presented in x, y and z axes. Then, ANOVA was followed using either Moderated T-tests or two-way ANOVA depended on the interpretation, and finally fold change analysis as statistical tools. A cut-off value of $P < 0.05$ (default) was considered statistically significant in ANOVA using the Benjamini and Hochberg False Discovery Rate set to 5% for multiple testing corrections. *P* value can be adjusted developing a better understanding of how the *P* value cut-off affects the results. The larger the *P* value the larger metabolite list arises. Statistical evaluation was completed with fold change analysis providing significant up or down metabolite regulations. Such small sample data sets can result in *P*-values of questionable statistical validity. For that purpose, the fold-change setting was used as a further filtering to look for significant differences. Basically, compounds were further filtered based on their abundance ratios that were greater than a specified cut-off value of 2. Fold change may be calculated between two conditions where both are treated as a single group and may also be referred to as a parameter value or an attribute value. In this study, fold change was calculated using normalized abundance evaluating the absolute difference between the normalized intensities of the conditions (FC = Condition 1 – Condition 2). All metabolites were identified using METLIN (a metabolite database used in metabolomics) however; they were not verified by authentic standards.

3.6.3.2. Sample extraction

Freeze-dried potato flesh and heart tissue samples (50 mg) (Fig. 3.14a) were powdered using a grinder (Restch Gmbh, Haan, Germany) and weighed in 2 ml microtubes (Eppendorf type polypropylene microtubes, Deltalab, Rubi, Barcelona, Spain) (Fig. 3.14b) and extracted with 1.5 ml of 50:50 HPLC grade methanol:water (v/v) + 1% formic acid (HCOOH). Subsequently, the supernatant was filtered through a 0.2 μm filter (Cronus PTFE

filters, Jaytee Biosciences Ltd., Kent, UK) driven by a 3 ml syringe (Fig. 3.14c) and stored at -40^{o}C until analysis.

3.6.3.3. Liquid Chromatography Mass Spectrometry (LC/MS) parameters

Samples (10 μl injection) were run using an ElectroSpray Ionisation (ESI) source in positive and negative mode on an Agilent Technology 1290 Infinity UPLC coupled with Agilent Technologies 6540 GHD Accurate-Mass Quadrupole Time of Flight (Q-ToF) mass spectrometer (Fig. 3.11). Chromatography was performed on a WATERS Acquity UPLC column CSH C_{18} 1.7 μm 2.1 x 150mm column (Ireland, UK) column with a gradient of eluent A: 0.1% (v/v) formic acid for LC/MS in HPLC grade water and eluent B: acetonitrile for LC/MS + 0.05% formic acid for LC/MS. Flow rate was set at 0.4 ml/min. The mobile phase was as follows: time 0 min, 95% A, 5% B; 5 min, 95% A, 5% B; 15 min, 10% A, 90% B; 15.10 min 100% B; 18.10 min, 100% B; 18.15 min, 95% A, 5% B; 21 min, 95% A, 5% B. Run time per sample was 21 min.

3.7. Gas diffusivity experiment (Year 3, 2013-2014)

A gas diffusivity experiment of two potato stocks cv. Maris Piper with different susceptibility to blackheart (BH) disorder [one susceptible stock (stock 10) and one non-susceptible (stock 4)] was conducted from the 30th of May 2014 till 13th of June 2014 at the Faculty of Bio-science Engineering (K.U.Leuven) in Leuven (Belgium). 40 potato tubers in total (20 tubers / stock) were delivered by courier on the 27th of May 2014 from Sutton Bridge Crop Storage Research (SBCSR, UK) to Leuven within four days. Potato tubers that were packed in Kraft paper bags directly placed on plastic trays and stored at 4^{o}C pending further processing. Tubers were initially stored at 3.5^{o}C for ca. 8 months at SBCSR.

This experiment was divided in two parts. First part of experiment was the micro- and nano-computed tomography scanning (micro-CT and nano-CT respectively) using x-rays that carried out on the 28th & 29th of May and 6th of June 2014 respectively at the Department of Metallurgy and Materials Engineering (MTM/MME, K.U.Leuven). Seven tubers were used for scanning in total. The second part of the experiment was the O_2 diffusivity measurement which took place at the Faculty of Bio-science Engineering (K.U.Leuven). Potato tuber volume and density were recorded. Respiration rate of tuber disks was also measured and used for O_2 diffusivity calculation. 10 tubers per stock were used in total.

3.7.1. Sample preparation before micro- and nano-computed tomography scanning

On the 28th of May 2014 micro-CT scanning test was carried out using micro-CT Skyscan instrument (Skyscan 1172, Brucker, Belgium) (Fig. 3.17). One potato tuber from stock 4 (non-susceptible to BH) was randomly selected and prepared for scanning. One 10 mm in thickness tuber slice was cut longitudinally with a sharp knife and then a cylinder was cut using a small cork borer (3 mm in diameter) (Fig. 3.18a). The cylinder was further cut in half (5 mm in thickness) and rolled with parafilm in order to prevent any sample dehydration. Then the sample was fitted into a cylinder shaped plastic holder and placed onto the scan stage for rotation (Fig. 3.18b). Scans were initially set at 1.4 μm, but increased to 2 μm for better image definition. Scanning time was ca. 75 min / sample.

After scanning test, on the 29th of May 2014 four (4) tubers in total (2 tubers / stock) were taken out from storage at 4°C and used for micro-CT scanning. One flesh and one heart tissue sample from each tuber were scanned (8 samples in total) (Fig. 3.18a). Flesh tissue samples were mainly taken from the perimedullary zone of the tuber, whilst heart tissue samples derived from the pith area (Fig. 3.18a). After scanning, data was collected and

pictures were reconstructed using NRecon RECONSTRUCTION software and further converted to binary images (500 images / sample) using CTAn (v.1.14.4) + CTVol (v.2.2) software and used for three-dimensional (3D) microstructure modelling.

Figure 3.17 SkyScan 1172 High Resolution Micro-CT instrument (Brucker, Belgium).

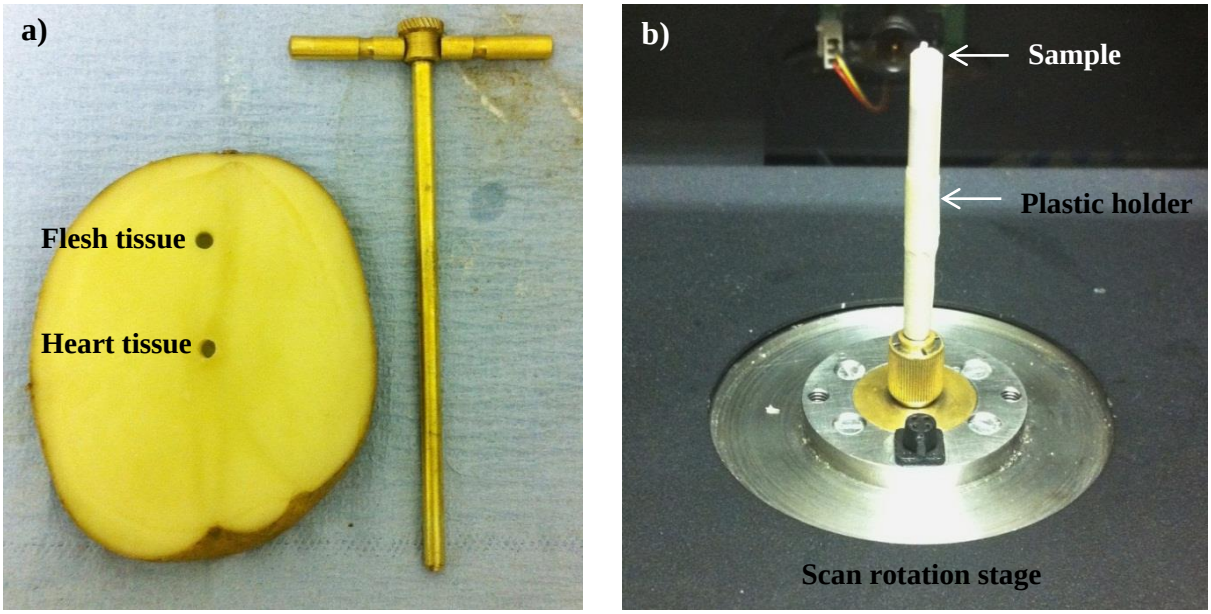


Figure 3.18 Tuber slice and cork borer (3 mm in diameter) (a) and scan rotation stage with parafilm covered sample on the top (b).

Nano-CT scanning was carried out using a Phoenix nanotom S system (GE Measurement & Control, USA) (Fig. 3.19). One potato tuber of stock 10 (susceptible to BH) and one from stock 4 (non-susceptible to BH) were randomly selected and used for scanning.

One slice 10 mm in thickness was cut longitudinally using a sharp knife. Then three heart tissue samples in cube shape (ca. 2 x 2 x 3 mm) were further cut using a razor blade. The scanning time was 60 min for the first heart sample, 90 min for the second and 180 min for the third one. However, nano-CT scans were unsuccessful due to the long scanning time and also the high radiation applied.

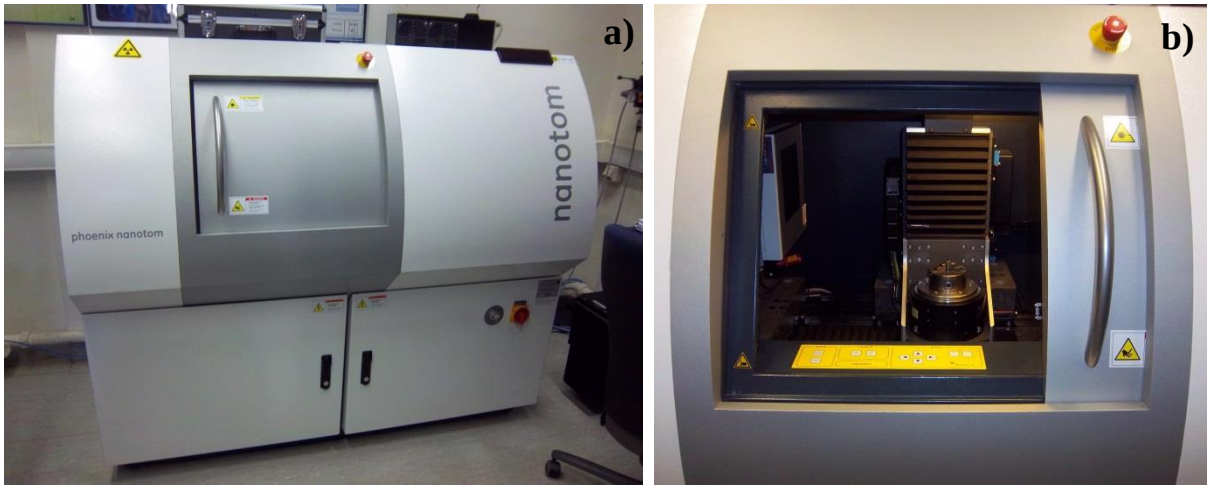


Figure 3.19 Phoenix nanotom S nanofocus computed tomography (nano-CT) instrument (a) and sample scan stage (b).

3.7.2. Sample preparation for respiration rate measurement

Two potato tubers per stock [stock 10 (susceptible to BH) and stock 4 (non-susceptible to BH) were randomly selected and taken out from storage at 4^{o}C. Each tuber was cut in half (Fig. 3.20b) and 5 mm in thickness slices (Fig. 3.20c) were further cut using an electric professional slice cutter (EH 158-L, Graef, Germany) (Fig. 3.20a). Afterwards, disk shaped samples with 24 mm diameter were taken using a cork borer (Fig 3.20d) and ca. 60 g were weighed using an electronic balance (Sartorius, surrey, UK) (Fig. 3.20e). Then disks were placed on wire trays and fitted in 1.8 L glass jars (Fig. 3.20f and g). Three jars per stock were used as replicates. The samples were incubated for ca. 1.5 h at 20^{o}C (baseline) (Fig. 3.20h)

and then O_2 and CO_2 concentrations were measured using the Checkmate II gas analyser (PBI Dansensor A/S, Ringsted, Denmark) (Fig. 3.21).The apparatus was equipped with a needle 8 mm in diameter The internal pressure of each jar was also measured with a pressure sensor (DPI 142, GE, Druck). After baseline measurement, the jars were placed back in the incubator and remained there for 18 h at 20^{o}C (Fig. 3.20h). The O_2 and CO_2 percentages were converted to partial pressures by multiplying with the internal pressure of the jar.

3.7.2.1. Respiration rate

Respiration rate in terms of O_2 consumption (R_{O2}) was calculated using the equation (Quang Tri Ho, personal communication, K.U. Leuven):

$$R_{O2} = \Delta p * V_{free} / V_{potato} * \Delta t * R * T \text{ (mol m}^3 \text{ s}^{-1}\text{)}$$

Δ**p** = partial pressure difference (kPa)

Vfree = Vjar – Vpotato (m^3)

Vpotato = tuber density / tuber mass (m^3)

Δ**t** = time difference (sec)

R = universal gas constant = 8.314 J mol^{-1} K^{-1}

T = temperature (K)

CO_2 production (R_{CO2}) was also calculated using a similar equation (formula not shown). The calculated tuber densities were averaged (n = 3) and used for the R_{O2} equation. The densities averaged for those tubers of stock 10 (susceptible to BH) and those of stock 4 (non-susceptible to BH) were 1.095 and 1.075 g ml^{-1} respectively. Respiration rate measurement of potato tuber disk samples from stock 10 (susceptible to BH) and stock 4 (non-susceptible to BH) were recorded after 1.5 h (baseline) and 18h at 20^{o}C and results

tabulated in Table 3.4 and 3.5 below. O_2 consumption measurements were further used for O_2 diffusion calculation later.

Table 3.4 O_2 consumption (R_{O2}) and CO_2 production (R_{CO2}) of potato disk samples from stock 10 (susceptible to BH).

					After 1.5 h			After 18 h								
Rep.	V jar (m^3)	Pot. disks weight (Kg)	Density (Kg/m^3)	Vpot (m^3)	O_2 (%)	CO_2 (%)	p (kPa)	O_2 (%)	CO_2 (%)	p (kPa)	Δt (sec)	T (K)	R_{O2} (mol m^3 s^{-1})	Average	R_{CO2} (mol m^3 s^{-1})	Average
1	0.001683	0.05837	1095	5.33E-05	20.5	0.2	101.75	17.2	3.1	101.07	69000	293	0.000631		0.000607	
2	0.001697	0.05976	1095	5.45E-05	20.4	0.3	101.65	16.6	3.8	99.72	69000	293	0.000748		0.000733	
3	0.001683	0.0647	1095	5.90E-05	20.2	0.3	100.9	15.9	4.2	99.97	69000	293	0.000733	0.000704	0.000736	0.000691

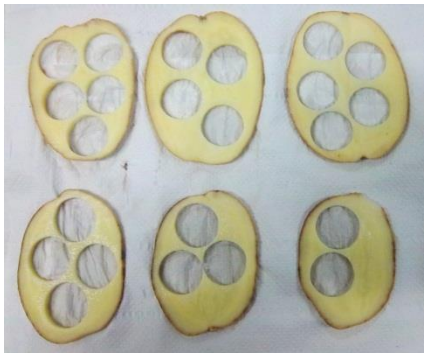
Table 3.5 O_2 consumption (R_{O2}) and CO_2 production (R_{CO2}) of potato disk samples from stock 4 (non-susceptible to BH).

					After 1.5 h			After 18 h								
Rep.	V jar (m^3)	Pot. disks weight (Kg)	Density (Kg/m^3)	Vpot (m^3)	O_2 (%)	CO_2 (%)	p (kPa)	O_2 (%)	CO_2 (%)	p (kPa)	Δt (sec)	T (K)	R_{O2} (mol m^3 s^{-1})	Average	R_{CO2} (mol m^3 s^{-1})	Average
1	0.001728	0.05864	1075	5.45E-05	20.3	0.4	99.92	16.1	4.1	99.53	77580	293	0.000691		0.000727	
2	0.001673	0.0585	1075	5.44E-05	20.4	0.3	100.98	16.3	4	99.54	77580	293	0.000688		0.000674	
3	0.001673	0.05951	1075	5.54E-05	20.2	0.3	100.84	16.1	4.2	99.98	77580	293	0.000660	0.000680	0.000696	0.000699

a) Slice cutter

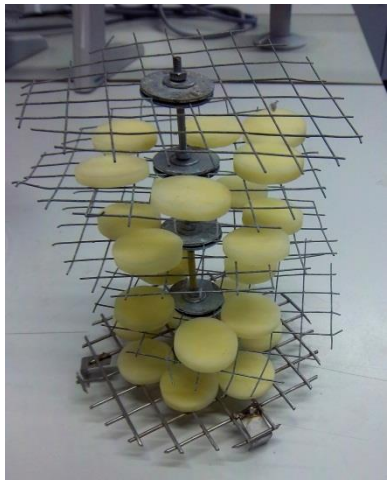
b) Tuber slicing

c) 5mm in thickness tuber slices



d) 24 mm in diameter disk shaped samples

e) 60 g sample weight



f) Samples on wire tray

g) Glass jar

h) Jars in the incubator at 20^{o}C

Figure 3.20 Sample preparation before respiration rate measurement.

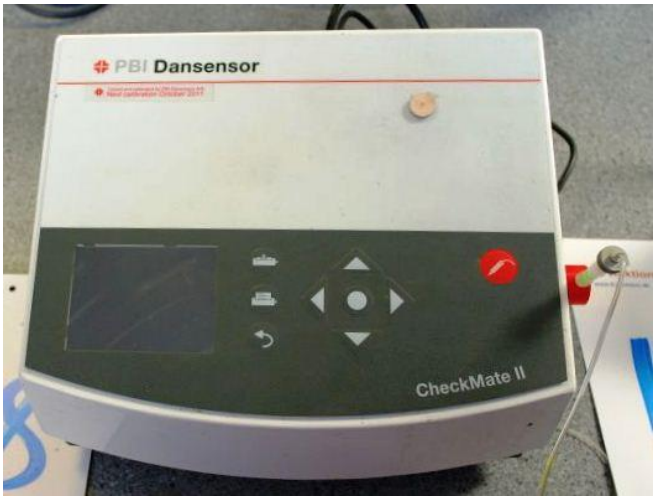


Figure 3.21 PBI Checkmate II gas analyser.

3.7.3. Sample preparation for O_2 diffusivity measurement

Initially, each potato tuber was cut in half longitudinally with a sharp knife (Fig. 3.22a). A cylinder shaped sample was taken radially using a cork borer (24 mm in diameter) and further cut into smaller cylinders using a razor blade (Fig 3.22a). The thickness of the samples was measured with a digital calliper (Mitutoyo Ltd, Hampshire, UK) and ranged from 1.99 to 2.85 mm. The samples were dried using roll paper and then each one was glued on a polyvinyl chloride (PVC) ring with cyano-acrylate glue (Superglue®, Loctite-Henkel, Brussels, Belgium) (Fig. 3.22c). Also, a rubber O-ring was fitted on the PVC ring (Fig. 3.22b). Then, a petroleum jelly based product (Vaseline) was applied on the rings sideways.

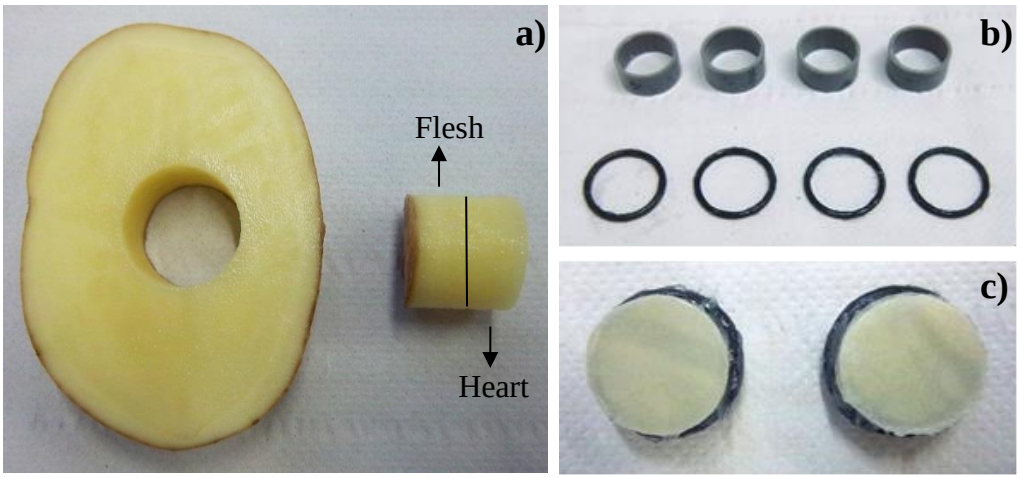


Figure 3.22 Example of potato tuber sampled and cylinder shaped samples (a). PVC and rubber rings (b). Disk shaped tissues attached on PVC rings covered with vaseline sideways (c).

3.7.4. Diffusion system set-up

O_2 diffusivity was measured using a diffusion system consisted of two metal chambers (measurement chamber and flushing chamber) (Fig. 3.23a) separated from a disk shaped potato tissue sample. The chambers were screwed together holding a PVC ring with a tissue sample attached on it. Also, a rubber O-ring was fitted on the PVC ring for perfect sealing between the chambers. Both rings were covered with vaseline to avoid any gas leakage and to ensure that gas was transported only through the sample. Once the sample was attached in the diffusion system then tubing connection took place between chambers and pressure sensors. The chambers were also connected together with tubes (Fig. 3.23b). Two inlet and outlet gas channels were used for flushing the gases into the measurement chamber and the flushing chamber. Pressure changes in each chamber were monitored using pressure sensors (PMP 4070, Dimed N.V., Antwep, Belgium).

Figure 3.23 Diffusion chambers before (a) and after set-up (b).

Before each measurement, the O_2 sensor was calibrated at 5, 20 and 30 kPa O_2 for ca. 30 min. The flushing chamber was always kept at 5 kPa of O_2 (Fig. 3.24). After calibration, the in- and out- valves (green tubes) of the measurement chamber were closed to stop flushing and the decrease in O_2 partial pressure and total pressure of the measurement chamber were recorded for ca. 3 h (Fig. 3.24). The flow rate used in both chambers was set at 10 L h^{-1}. After measurement, the in- and out- valves were opened and re-calibration was followed at 30, 20 and 5 kPa O_2 (Fig. 3.24). The O_2 concentration was measured in the measurement chamber using fluorescent optical probes (Foxy-Resp, Ocean Optics, Duiven, The Netherlands). This principle was based on fluorescence quenching of a ruthenium compound (Ru) by O_2 which diffuses into a dye covering the optic tip of the fibre optic probe.

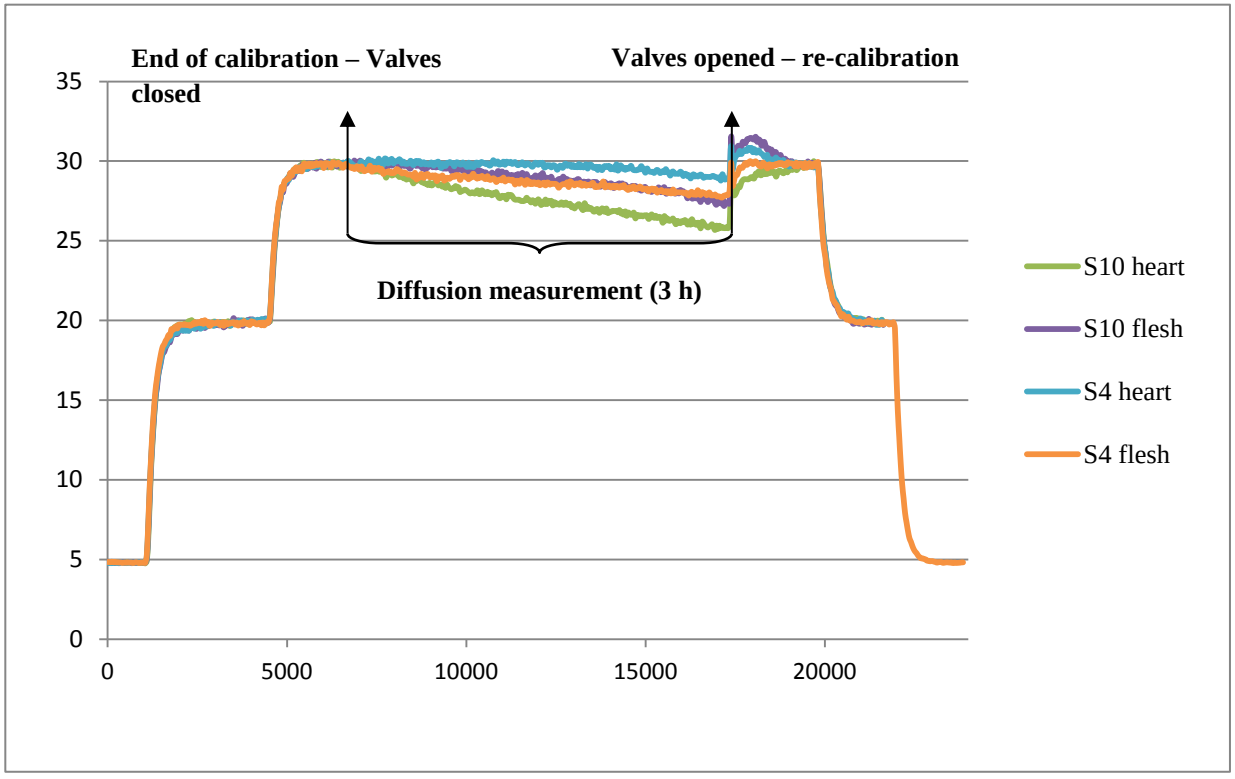


Figure 3.24 Example of O_2 calibration and diffusion measurement curve of flesh and heart tuber tissue of stock 10 (susceptible to BH) and stock 4 (non-susceptible to BH). Recording time (sec) and O_2 concentration (kPa) are shown on x and y axis respectively.

3.8. Statistical and multivariate analysis

Analysis of variance (ANOVA), two- sample T-tests, Principal Component Analysis (PCA; an unsupervised multivariate technique) and General Linear Model (GLM) were performed with GenStat 16th Edition (VSN International Ltd, Herts., UK). All metabolomics data was statistically analysed using Agilent Mass Profiler Professional (MPP) software. The statistical tools used were moderated T-test, two-way ANOVA and PCA analysis.

General Analysis of variance (ANOVA) was used in order to estimate the interactions between the levels of factors on CO_2 production of potato tubers to a significance of $P < 0.05$ in both years (1 and 2). Sugar and phenolic data sets were also analysed by general ANOVA. Least significant differences (LSD; $P = 0.05$) were also calculated from each data analysis. Two sample t-tests were used in order to find significant differences between respiration rate, tuber length, tuber diameter and tuber size with the blackheart incidence respectively and one-sided ($y<0$) correlations were further used when needed.

General Linear Model (GLM) was used in the 2nd experiment of year 2 (2012-2013) in order to identify significant differences in blackheart incidence between those tubers stored under various gas combinations on five sampling days (section *3.2.2.2*). PCA was used for a better visual representation by clustering groups of data sets analysed after sugar, phenolic and untargeted metabolomics analysis.

Moderated T-test and two-way ANOVA were applied on untargeted metabolomics data sets in order to identify significant differences in metabolite abundance to a significance of $P < 0.001$, 0.01, 0.02 or 0.05 depended on the interaction between the parameters analysed.

CHAPTER FOUR:

The effect of storage temperature and shelf-life conditions on the physiological and biochemical changes of potato stocks with different susceptibility to blackheart disorder (Year 1: 2011-2012)

4.1. Introduction

Blackheart disorder (BH) is a physiological internal disorder induced in potato tubers resulting in brown to black tissue discoloration (severity depended) and cell necrosis. It has previously been proposed that this disorder is caused due to O_2 depletion and/or CO_2 accumulation in the central tuber tissues at any temperature. BH disorder is considered as a severe problem for potato packing industry causing economic losses to the markets as tissue discoloration becomes apparent when tubers are sliced open. However, it is still unclear how this disorder is induced and which pre- and post-harvest factors contribute to its development. In this study, an attempt to mimic shelf-life conditions was evaluated by initially storing potato tubers of Maris Piper stocks with different susceptibility to BH at very low temperatures (1.5 or 3^{o}C) and then transferring them in air and/or CA storage (10% CO_2) at 15^{o}C for shelf-life evaluation. The aim of this study was to discover physiological and biochemical changes in potato tubers with different susceptibility to BH.

4.2. Materials and methods

Sample preparation for respiration rate measurement, non-structural carbohydrate content (sugars) and phenolic content analysis were described in Chapter 3; Sections 3.4, 3.5 and 3.6.

4.3. Respiration rate

Potato tuber respiration rate was recorded after baseline (sampling days 0, 3 and 7) and at outturn 2, 3, 4 and 5 on sampling days 0, 3 and 7 and studied at 15^{o}C. After baseline, tubers were initially stored at 1.5 or 3^{o}C for 8, 12, 16 and 20 weeks. Statistical analysis of results was performed using variance analysis (ANOVA) in order to estimate the interactions between the levels of factors on CO_2 production of potato tubers. These factors included stock [stock 20 (susceptible to BH), stock 23 [(susceptible to BH) and stock 12 (non-susceptible to BH)], storage condition (air or CA), initial storage temperature (1.5 or 3^{o}C), outturns (baseline included), sampling days (0, 3 and 7) and replication. Interactions of the above factors were statistically significant in particular combinations (Table 4.1). It is worth noted that efflux of CO_2 was included in respiration rate for those tubers held under 10% CO_2 and O_2 consumption was not calculated during this experiment.

According to Figure 4.1, a similar trend in respiration rate was followed over storage time. That was an increase in respiration rate on sampling day 3 and then a slight decrease on sampling day 7 (Fig. 4.1). Generally, stock 23 (susceptible to BH) recorded the lowest respiration rate when compared to stock 20 (susceptible to BH) and stock 12 (non-susceptible to BH). Tubers stored under CA (10% CO_2) had a greater respiration rate compared to those stored in air only and that was due to the CO_2 efflux. Moreover, respiration rate was higher in those tubers initially stored at 1.5^{o}C compared with those at 3^{o}C and this was more pronounced in CA conditions (Fig. 4.1). However, there was a variation in respiration rate between the stocks analysed over storage time.

It should be noted that respiration rate measurement was carried out at 15^{o}C during the whole experiment. On sampling day 0, stock 12 (non-susceptible to BH) had ca. 2- and 3-times higher respiration rate compared to stock 20 (susceptible to BH) and stock 23

(susceptible to BH) (6.6, 4.2 and 2.25 ml CO_2 kg^{-1} h^{-1} respectively). On sampling day 3 after storage in air only, respiration rate was significantly reduced for those tubers of stock 12 and stock 23, but significantly increased 2-fold for stock 20 (3.7, 3.7 and 6.4 ml CO_2 kg^{-1} h^{-1}) (Fig. 4.1a). After storage in CA on day 3, comparison between the stocks showed that stock 20 had the highest CO_2 concentration (15.1 ml CO_2 kg^{-1} h^{-1}) and stock 23 the lowest (9.3 ml CO_2 kg^{-1} h^{-1}) (Fig. 4.1). On sampling day 7 in air storage, the CO_2 rate of stock 12 significantly increased (5.5 ml CO_2 kg^{-1} h^{-1}) but remained steady for stock 20 (6.1 ml CO_2 Kg^{-1} h^{-1})) and stock 23 (3.8 ml CO_2 kg^{-1} h^{-1}) compared with those rates recorded on day 3 (air storage). Also, on day 7 only (CA), comparison between the stocks showed that stock 23 had the lowest CO_2 rate compared with stock 12 and stock 20 (8.6, 11.2, 13.4 ml CO_2 kg^{-1} h^{-1} respectively). In general, the CO_2 production after CA storage in both sampling days 3 and 7, were 2- to 3 –fold higher compared to storage in air only due to CO_2 efflux (Fig 4.1).

For a better visualization and interpretation of the results, respiration rate graphs were separated and visualized according to stock per sampling day (0, 3 or 7) per storage condition (air or CA) after storage at 1.5 or 3^{o}C over storage time as respiration rate was always significant higher in CA compared to air storage over storage time (Fig. 4.2A and 4.3A). Alternative visualization of respiration rates recorded after air and CA storage conditions are shown in Figure 4.2B and 4.3B, respectively.

Briefly, on each sampling day 0 (air only) there were no significant differences between the stocks at outturn 2, 3 and 4 namely after initial storage at very low temperature for 8, 12 and 16 weeks. After 8, 12 and 16 weeks of cool storage at 1.5 or 3^{o}C, the mean CO_2 rates recorded on sampling days 0 at 15^{o}C were 2.62 and 2.56 ml CO_2 kg^{-1} h^{-1} for stock 20 (susceptible to BH), 2.88 and 2.39 ml CO_2 kg^{-1} h^{-1} for stock 23 (susceptible to BH), 3.52 and 2.8 ml CO_2 kg^{-1} h^{-1} for stock 12 (non-susceptible to BH) respectively. Comparing air storage only, on sampling day 3 respiration rate produced was ca. 2- to 4-times higher (stock

depended) compared to those rates recorded on sampling day 0 and 2- to 3-times higher for those tubers initially stored at 1.5^{o}C when compared with those initially stored at 3^{o}C. On each sampling day 7, it was observed that respiration rate was lower for those tubers initially stored at 1.5^{o}C than 3^{o}C for 8 and 12 weeks of storage. In contrast, after 16 and 20 weeks of cold storage, respiration rate was higher for initial storage at 1.5 compared to 3^{o}C. A similar trend in respiration rate between the stocks was not really distinguished due to large or small variation in CO_2 rates recorded after sampling days 3 and 7. In terms of the CA storage (10% CO_2), it seemed that those tubers initially stored at 1.5^{o}C showed higher respiration rate compared with those initially stored at 3^{o}C recorded on sampling days 3 and 7, but still a similar pattern in CO_2 production between those stocks analysed was not distinct due to variation in respiration rate recorded on those sampling days.

Table 4.1 Interactions of the factors on potato tuber respiration rate (Appendix B; Table 1).

Factor	d.f.	s.s	m.s	v.r.	F pr.
Stock (1)	2	215.258	107.629	32.26	< 0.01
Condition (2)	1	3453.628	3453.628	1035.26	< 0.01
Temperature (3)	2	573.517	286.759	85.69	< 0.01
Days (4)	2	0.101	0.051	0.02	0.440
Outturn (5)	6	75.072	12.512	3.75	0.001
Interactions					
1 x 2	2	87.172	43.586	13.07	< 0.01
2 x 5	3	137.224	45.741	13.71	< 0.01
4 x 5	3	84.875	28.292	8.48	< 0.01
2 x 4 x 5	3	43.565	14.522	4.35	0.005
3 x 4 x 5	3	68.210	22.737	6.82	< 0.01
1 x 2 x 3 x 4 x 5	6	7.996	1.333	0.40	0.765

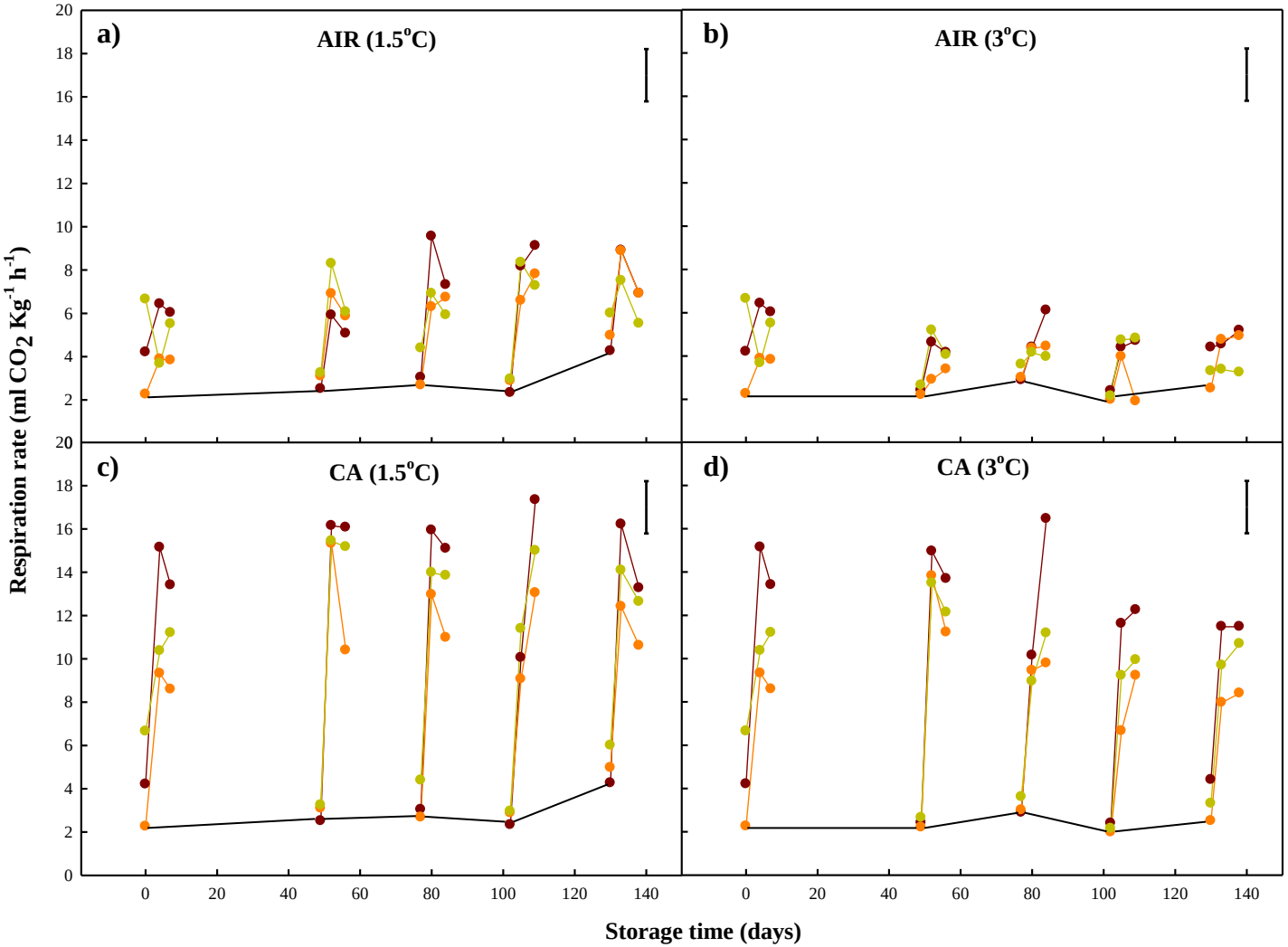


Figure 4.1 Respiration rate (ml CO_2 kg^{-1} h^{-1}) of potato cv. Maris Piper stock 20 [brown (susceptible to BH)], stock 23 [orange (susceptible to BH)] and stock 12 [green (non- susceptible to BH)] recorded after baseline, outturn 2, 3, 4 and 5 at 15°C on sampling days 0 (air only)*, 3 (air or CA)**, and 7 (air or CA)**. Tubers were initially stored at 1.5 or 3°C for 8, 12, 16 and 20 weeks. Values are means (n = 3).General LSD is shown. [*air (O_2 = 21%), **CA (O_2 = 18-19%, CO_2 = 10%)]. Black line (-) shows all sampling days 0.

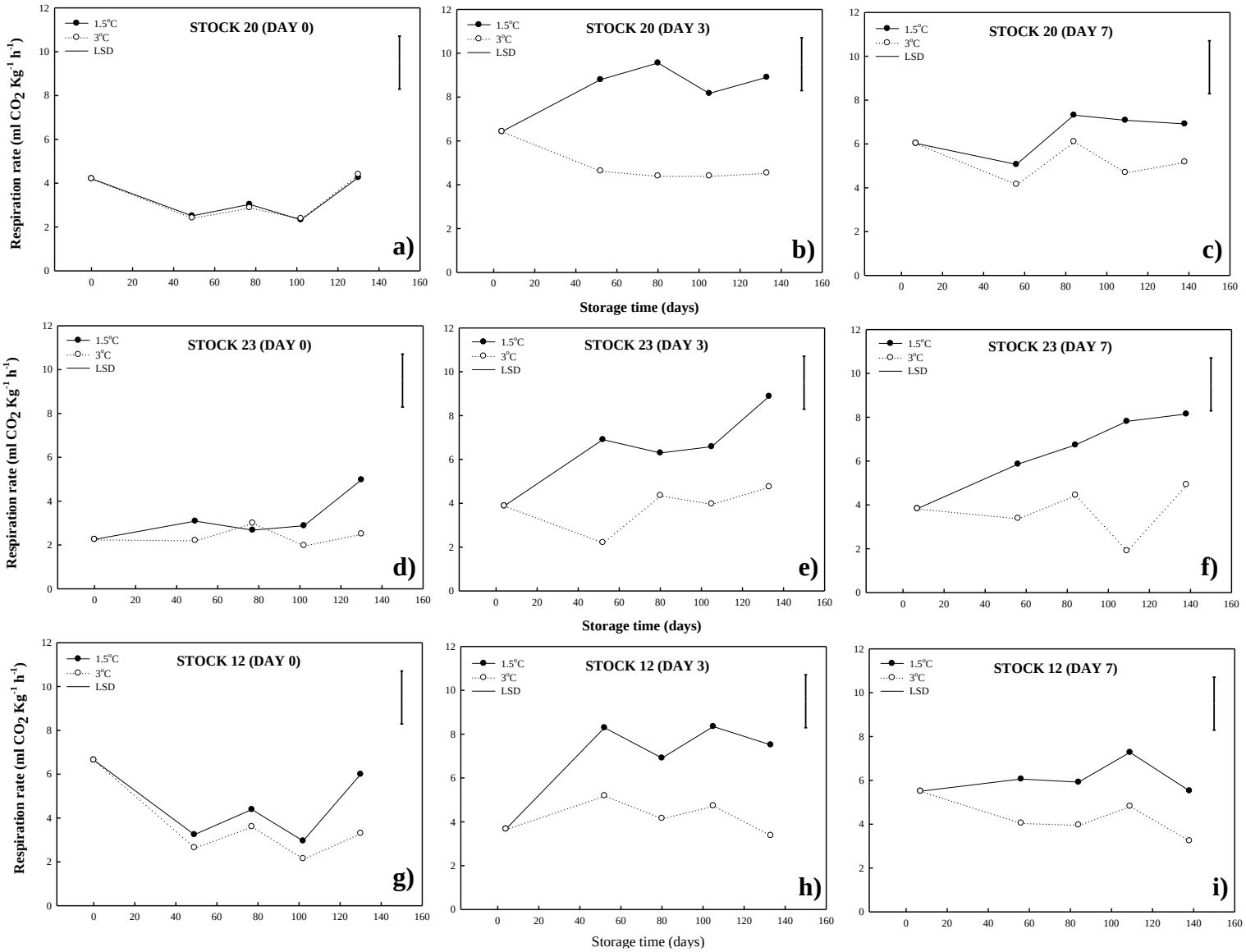


Figure 4.2A Respiration rate (ml CO_2 kg^{-1} h^{-1}) of potato cv. Maris Piper stock 20 (susceptible to BH), stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) recorded after baseline, outturn 2, 3, 4 and 5 at 15°C in air only on sampling days 0, 3 and 7. Tubers were initially stored at 1.5 or 3°C for 8, 12, 16 and 20 weeks. Values are means (n= 3). General LSD is shown.

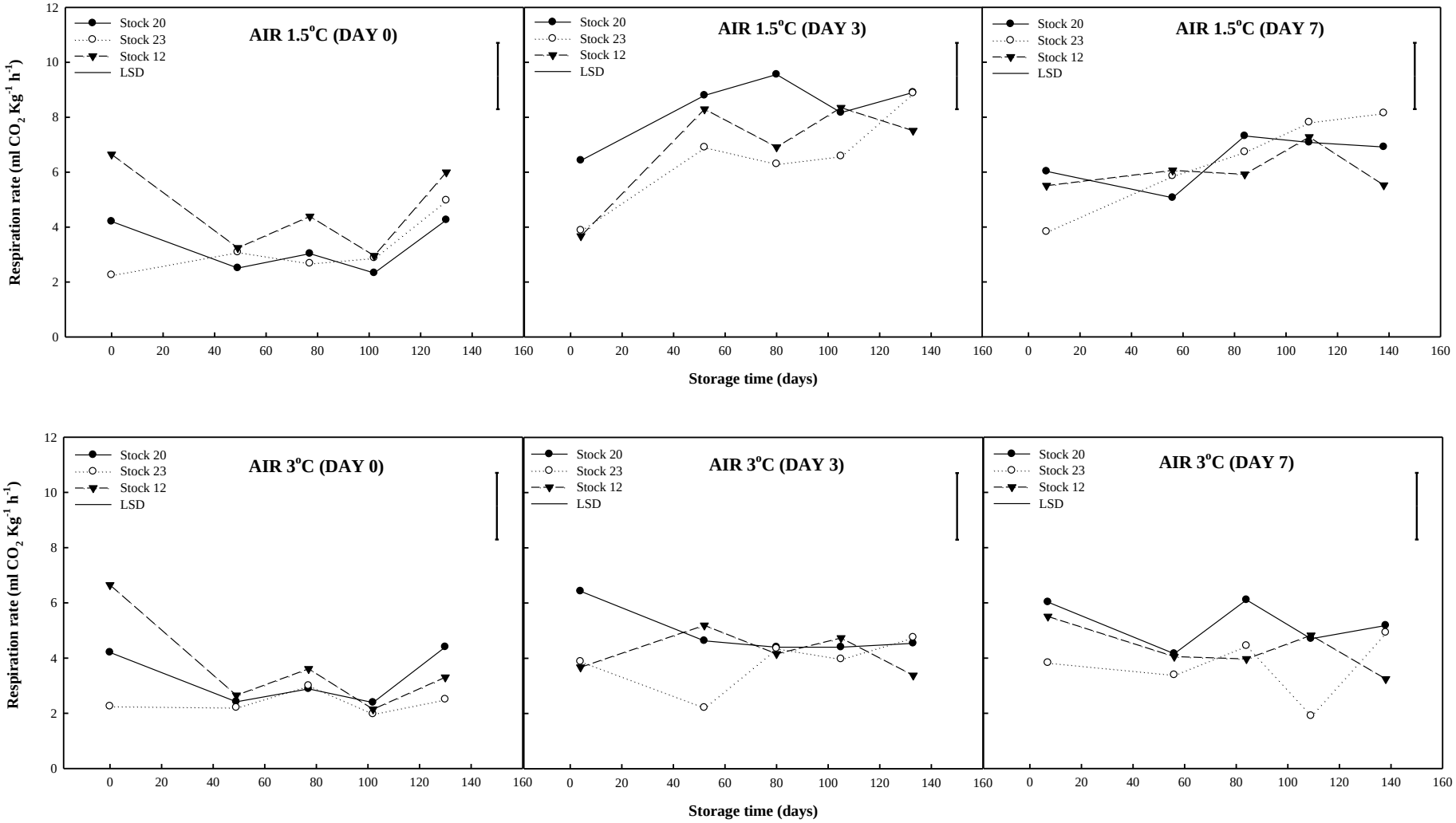


Figure 4.2B Alternative visualization of respiration rate (ml CO_2 kg^{-1} h^{-1}) of stock 20 (susceptible to BH), stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) recorded over storage time in air only at 15°C on sampling days 0, 3 and 7. Initial storage temperature and general LSD is shown.

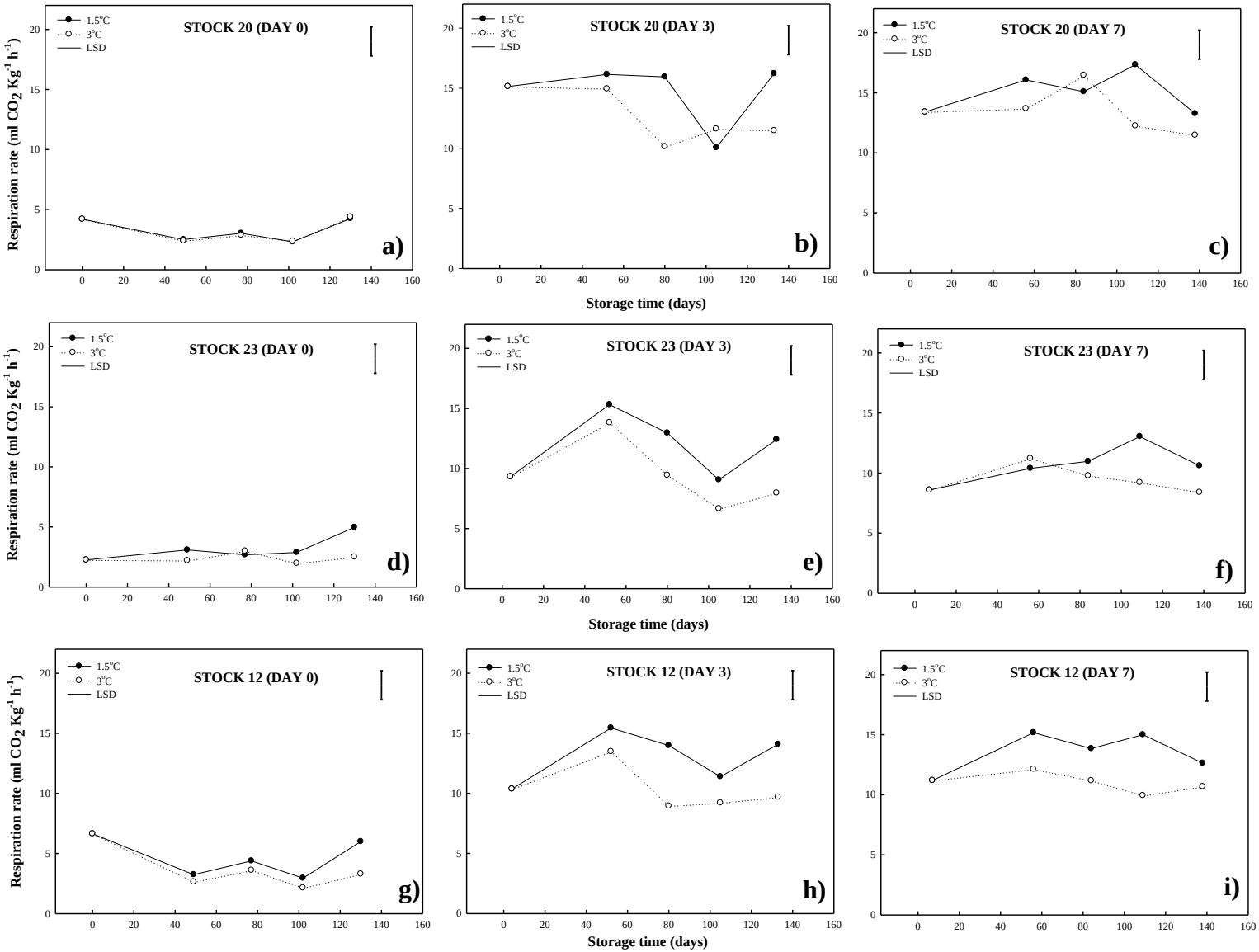


Figure 4.3A Respiration rate (ml CO_2 kg^{-1} h^{-1}) of potato cv. Maris Piper stock 20 (susceptible to BH), stock 23 (susceptible to BH) and stock 12 (non- susceptible to BH) recorded after baseline, outturn 2, 3, 4 and 5 at 15°C on sampling days 0 (air only)*, 3 (CA)**, and 7 (CA)**. Tubers were initially stored at 1.5°C for 8, 12, 16 and 20 weeks. Values are means (n= 3). General LSD is shown. [*air (O_2 = 21%), **CA (O_2 = 18-19%, CO_2 = 10%)].

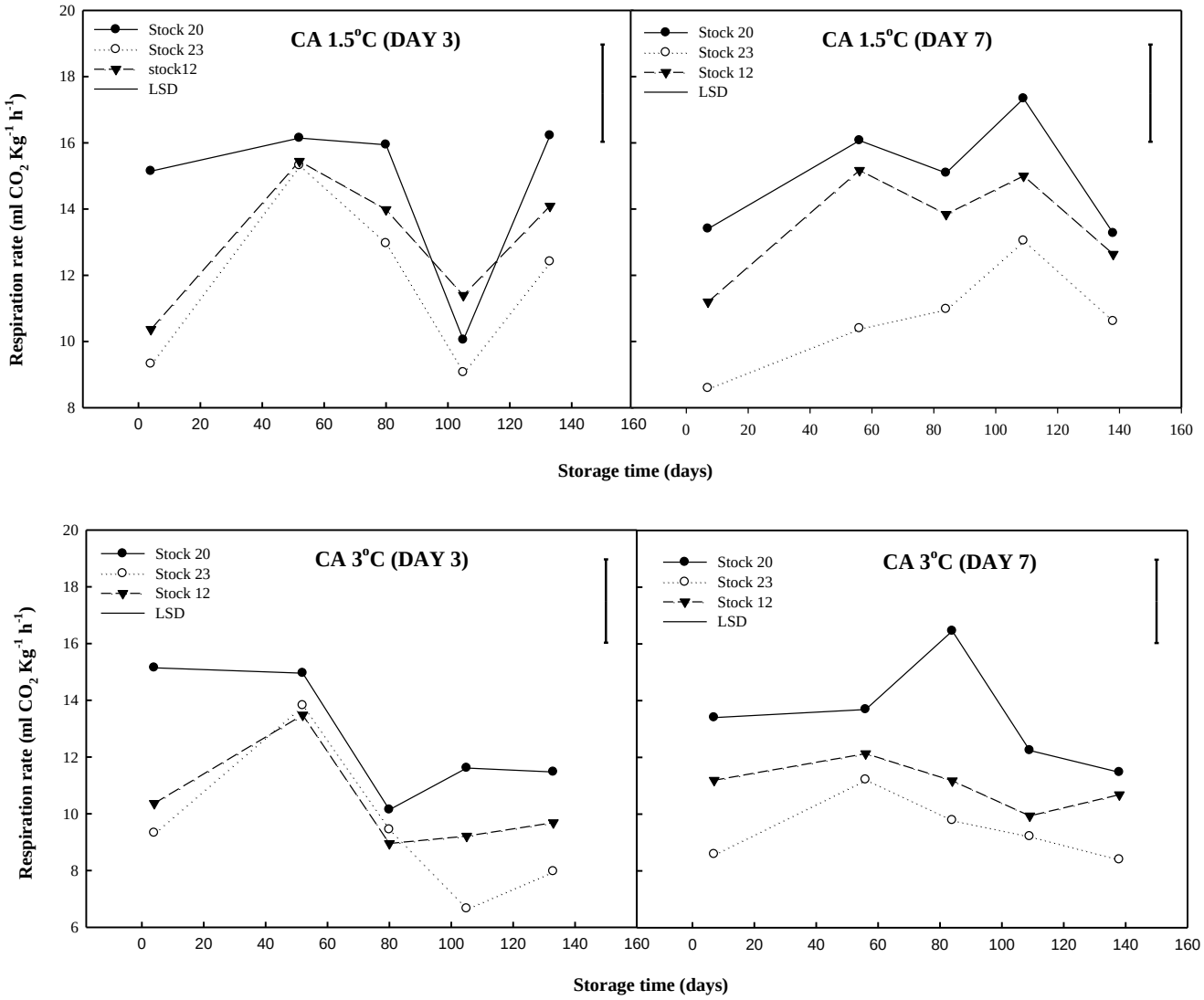


Figure 4.3B Alternative visualization of respiration rate (ml CO_2 kg^{-1} h^{-1}) of stock 20 (susceptible to BH), stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) recorded after storage in CA (10% CO_2) at 15°C on sampling days 3 and 7 over storage time. Initial storage temperature and general LSD is shown.

4.4. The incidence of blackheart

BH incidence for potato tubers cv. Maris Piper stock 20, stock 23 (both susceptible to BH) and stock 12 (non-susceptible to BH) after 163 days storage in total are tabulated in Table 4.2. Tubers were sampled at 3 different sampling days per four weeks [*viz.* day 0 (air only), 3 (air or CA) and 7 (air or CA)] at 15^{o}C (n = 1512 tubers in total). No indications of discoloration were shown at baseline and after 27 days of storage (week 4). However, incidence of BH was initially observed after the 49th day of storage (8.1%). peaking in March (11.8%) and in May (15.5%) after 20 and 24 weeks of storage, respectively (Table 4.2). It should be noted that outturn 1 (4 weeks of storage) was conducted in air only. Also, the last outturn 6 (24 weeks of storage) was carried out with tubers that were initially stored at 1.5^{o}C only (n = 135). That happened because tubers that were initially stored at 3^{o}C became mouldy and had to be discarded. Table 4.3 shows the percentage of BH incidence for each stock separately per storage condition (air or CA), storage time and initial storage temperature. The incidence of BH was greater for stock 23 (susceptible to BH) at both storage conditions over storage time compared to stock 20 (susceptible to BH) and stock 12 (non-susceptible to BH) (Table 4.3). In particular, BH incidence was ca. 3 times greater in stock 23 compared to stock 20. Example figures of potato tubers showing tissue discolorations are presented in Appendix A; Figure 1.1 – 1.7.

However, in order to find any difference in BH susceptibility between those two susceptible stocks (stock 20 and 23) Chi Square tests were performed using GenStat 16th Edition (VSN International Ltd, Herts., UK). The susceptibility in BH was assessed at each storage condition (air or CA) per initial storage temperature (1.5 or 3^{o}C) separately over storage time from outturn 2 -in which tuber tissue discoloration was initially indicated- to outturn 5 (8 to 20 weeks of storage, respectively) (Table 4.4). Chi Square tests results

revealed that the susceptibility in BH was significantly higher in stock 23 compared to stock 20 in both storage conditions (air or CA) when initially stored in either 1.5 or 3^{o}C (Table 4.4).

Table 4.2 Total percentage of BH incidence over storage time.

Sampling point		Month	Year	Tubers used	BH incidence (%)
BL	Baseline (days 0, 3 and 7)	November	2011	135	0
Outturn 1	Week 4 (days 27, 30 and 34)	December	2011	162	0
Outturn 2	Week 8 (days 49, 52 and 56)	January	2012	270	8.1
Outturn 3	Week 12 (days 77, 80 and 84)	February	2012	270	5.2
Outturn 4	Week 16 (days 102, 105 and 109)	March	2012	270	11.8
Outturn 5	Week 20 (days 130, 133 and 138)	April	2012	270	5.9
Outturn 6	Week 24 (days 156, 159 and 163)	May	2012	135	15.5
	TOTAL			1512	9.6

Table 4.3 Percentage of BH incidence for potato cv. Maris Piper stock 20 (susceptible to BH), stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) after storage in air or CA at 15°C. Tubers were initially stored at 1.5 or 3°C for 8, 12, 16, 20 and 24 weeks.

BH (%)	AIR						CA					
Weeks of storage	Stock 20		Stock 23		Stock 12		Stock 20		Stock 23		Stock 12	
	1.5°C	3°C	1.5°C	3°C	1.5°C	3°C	1.5°C	3°C	1.5°C	3°C	1.5°C	3°C
Week 8	3.7	14.8	7.4	14.8	0	0	11.1	11.1	27.7	11.1	0	0
Week 12	7.4	3.7	11.1	14.8	3.7	0	5.5	5.5	0	5.5	0	0
Week 16	7.4	7.4	37	33.3	0	0	5.5	0	5.5	38.8	0	0
Week 20	3.7	3.7	25.9	18.5	0	0	0	0	5.5	5.5	0	0
Week 24	7.4	-	33.3	-	0	-	22.	-	33.3	-	0	-
TOTAL	5.9	7.4	22.9	20.3	0.7	0	8.8	4.2	14.4	15.3	0	0

Table 4.4 Chi-Square test results of BH incidence for potato tubers cv. Maris Piper stock 20 and stock 23 after storage in air at 15°C. Tubers were initially stored at 1.5 or 3°C for 8, 12, 16, 20 and 24 weeks ($P < 0.05$) (Appendix B; Table 2.1 – 2.4).

	AIR			
	BH (%) 1.5°C	Probability	BH (%) 3°C	Probability
Stock 20	5.5	0.001	7.4	0.005
Stock 23	20.3		20.3	
	CA			
	BH (%) 1.5°C	Probability	BH (%) 3°C	Probability
Stock 20	5.5	0.013	4.2	0.020
Stock 23	9.7		15.3	

4.4.1. Effect of tuber size and tuber weight on BH incidence

Potato tuber size was determined by measuring the length (mm) and the maximum equatorial diameter (mm) using a digital calliper. Tuber weight (g) was measured using a balance. Statistical analysis was performed with GenStat 16th Edition (VSN International Ltd., Herts., UK) using 'Two-sample' t-tests in order to estimate a possible link between the incidence of BH with the tuber size and weight respectively. Potato tubers with indications of BH or BH-like symptoms were initially detected after 8 weeks of storage According to Table 4.5, a significant effect of the tuber length ($P = 0.011$), equatorial diameter ($P = 0.016$) and weight ($P < 0.001$) on the BH incidence respectively, was observed in those tubers of stock 20 (susceptible to BH) which were initially stored at 1.5°C for 16 weeks and then stored in air at 15°C (Table 4.5). Affected tubers of stock 20 had greater length (mean = 104.90 mm), diameter (mean = 78.78 mm) and weight (mean = 313.20 g) than those that were unaffected. Moreover, weakly positive correlations were found between the BH incidence and the tuber length ($r = 0.44$), diameter ($r = 0.42$) and weight ($r = 0.58$), respectively (Table 4.6). There

was also a significant effect of the tuber length on BH incidence in those tubers of stock 20 (susceptible to BH) after storage in CA during week 8 (P = 0.034). Those tubers that were also derived from 1.5°C and had greater length (mean = 103.30 mm) than those which were unaffected (Table 4.7). However, no significant correlations were found regarding the tuber length and the BH incidence (Table 4.8). Regarding stock 23 (susceptible to BH), no significant effects of tuber length, equatorial diameter and weight on BH incidence were observed in those tubers of stock 23 (susceptible to BH) in both storage conditions (air or CA) and both initial storage temperatures (1.5 or 3°C) (Table 4.9 and 4.10).

Table 4.5 Two-sample t-test results of tuber length, diameter and weight with BH incidence for potato cv. Maris Piper stock 20 (susceptible to BH) after storage in air at 15°C. Tubers were initially stored at 1.5 or 3°C for 8, 12, 16 and 20 weeks ($P < 0.05$) (Appendix B; Table 3.1 – 3.12).

	1.5°C			3°C		
Sampling point	Length	Diameter	Weight	Length	Diameter	Weight
Week 8	-	-	-	0.805	0.552	0.743
Week 12	0.334	0.505	0.365	-	-	-
Week 16	0.011	0.016	< 0.001	0.326	0.255	0.284
Week 20	-	-	-	-	-	-

Table 4.6 Correlation matrix comparing the tuber length, diameter and weight with the BH incidence for potato cv. Maris Piper stock 20 after storage in air at 15°C. Tubers were initially stored at 1.5°C for 16 weeks ($P < 0.05$)* (Appendix B; Table 3.13).

	BH	Length (mm)	Diameter (mm)	Weight (g)
Blackheart	-	0.4378*	0.4155*	0.5765*
Length		-	0.4716*	0.8671*
Diameter			-	0.7298*
Weight				-

Table 4.7 Two-sample t-test results of tuber length, diameter and weight with the BH incidence for potato cv. Maris Piper stock 20 (susceptible to BH) after storage in CA at 15°C. Tubers were initially stored at 1.5 or 3°C for 8, 12, 16 and 20 weeks ($P < 0.05$) (Appendix B; Table 4.1 - 4.6).

	1.5°C			3°C		
Sampling point	Length	Diameter	Weight	Length	Diameter	Weight
Week 8	0.034	0.238	0.361	0.236	0.327	0.346
Week 12	-	-	-	-	-	-
Week 16	-	-	-	-	-	-
Week 20	-	-	-	-	-	-

Table 4.8 Correlation matrix comparing the tuber length, diameter and weight with the BH incidence for potato cv. Maris Piper stock 20 after storage in CA at 15°C. Tubers were initially stored at 1.5°C for 8 weeks ($P < 0.05$)* (Appendix B; Table 4.7)

	BH	Length (mm)	Diameter (mm)	Weight (g)
Blackheart	-	0.4402	0.1792	0.2620
Length		-	0.7400*	0.8173*
Diameter			-	0.8578*
Weight				-

Table 4.9 Two-sample t-test results of tuber length, diameter and weight with the BH incidence for potato cv. Maris Piper stock 23 after storage in air at 15°C.Tubers were initially stored at 1.5 or 3°C for 8, 12, 16 and 20 weeks ($P < 0.05$) (Appendix B; Table 5.1 - 5.24).

	1.5°C			3°C		
Sampling point	Length	Diameter	Weight	Length	Diameter	Weight
Week 8	0.227	0.498	0.480	0.614	0.331	0.247
Week 12	0.450	0.916	0.754	0.230	0.244	0.328
Week 16	0.775	0.672	0.659	0.414	0.083	0.118
Week 20	0.771	0.117	0.463	0.531	0.244	0.328

Table 4.10 Two-sample t-test results of tuber length, diameter and weight for potato cv. Maris Piper stock 23 after storage in CA at 15°C. Tubers were initially stored at 1.5 or 3°C for 8, 12, 16 and 20 weeks ($P < 0.05$) (Appendix B; Table 6.1 – 6.9).

	1.5°C			3°C		
Sampling point	Length	Diameter	Weight	Length	Diameter	Weight
Week 8	0.479	0.146	0.411	0.288	0.117	0.279
Week 12	-	-	-	-	-	-
Week 16	-	-	-	0.831	0.463	0.443
Week 20	-	-	-	-	-	

4.5. Biochemical assessments

4.5.1. Non-structural carbohydrate content analysis

Unsurprisingly, results revealed an increase in reducing sugar (fructose and glucose) accumulation over storage time due to the very low initial storage temperature (1.5°C). Although there was an increase through storage time, both sucrose and reducing sugar content varied among sampling days 0, 3 and 7 (Fig. 4.4). However, during baseline measurements there were no significant differences in fructose, glucose and sucrose content between the three stocks and yet between heart and flesh tissue samples. That was because no cold storage temperature applied at baseline. In general, it was observed that higher sugar content was accumulated more in the heart when compared to flesh tissue samples. Therefore, stock 23 (susceptible to BH) had greater sugar accumulation in comparison with stock 20 (susceptible to BH) and stock 12 (non-susceptible to BH).

4.5.1.1. Fructose

According to Figure 4.4a, the concentration of fructose was significantly increased in both tissue samples and yet accumulated more in the heart tissue for all three stocks after 8 weeks of storage. Flesh fructose of stock 20 (less susceptible to BH) and stock 12 (non-susceptible to BH) was slightly higher after 8 weeks of storage. However, after 16 weeks of storage, fructose increased almost 2-fold in both tissue samples of stock 20 (susceptible to BH) and stock 12 (non-susceptible to BH). Interestingly, stock 23 (susceptible to BH) accumulated the highest fructose content which was ca. 3 to 4 times higher after 8 and 16 and 20 weeks of storage compared to that measured at baseline before storage at cold temperature (Fig. 4.4a). The highest fructose concentrations were observed in heart tissue of stock 23 ranging from 103.9 to 130.4 mg g^{-1} DW.

4.5.1.2. Glucose

A similar pattern was observed for glucose whereby there was an increase in both tissue samples of all three stocks over storage time and no significant differences in glucose were indicated between the stocks at baseline regardless of tissue (Fig. 4.4b). Also, glucose content in flesh tissue of stock 20 (less susceptible to BH) had almost the same concentrations (36.9 to 67.4 mg g^{-1} DW) to stock 12 (non-susceptible to BH) (42.9 to 61.6 mg g^{-1} DW) from 8 to 20 weeks of storage. Once more stock 23 (susceptible to BH) had the greater glucose accumulation in both tissue samples and that was ca. 2-fold higher when compared to stock 20 and stock 12 after 8 and 16 weeks of storage. The highest glucose content was shown in flesh tissue (84.7 to 121.6 mg g^{-1} DW) and slightly higher in heart tissue (111.8 to 137.04 mg g^{-1} DW) of stock 23 after 16 weeks of storage (Fig. 4.4b). After 16 and 20 weeks of storage, glucose content was significantly higher in heart samples of stock 20 (ca. 76.8 mg g^{-1} DW) and stock 12 (68 mg g^{-1} DW) compared to their corresponding flesh samples (58.5 and 49.2

mg g^{-1} DW respectively). On the other hand, flesh and heart glucose content of stock 23 was significantly higher after 20 weeks of storage (77.6 and 98.7 mg g^{-1} DW respectively).

4.5.1.3. Sucrose

In terms of sucrose content, generally stock 23 (susceptible to BH) had significantly higher concentrations (mean = 21.79 mg g^{-1} DW) compared to stock 20 (susceptible to BH) (mean = 16.8 mg g^{-1} DW) and stock 12 (non-susceptible to BH) (mean = 17.6 mg g^{-1} DW). Figure 4.4c shows that flesh sucrose of stock 20 (susceptible to BH) was significantly higher (17.1 mg g^{-1} DW) compared to stock 23 (susceptible to BH) (14.1 mg g^{-1} DW) and stock 12 (non-susceptible to BH) (15 mg g^{-1} DW) after baseline measurements. Also at baseline, there were no significant differences in sucrose between flesh and heart tissue samples of stock 20 and stock 12, however sucrose content was significantly higher in heart samples (19.9 mg g^{-1} DW) of stock 23 and lower in flesh samples (14.1 mg g^{-1} DW) (Fig. 4.4c). After 8 weeks of storage, stock 23 had significantly higher sucrose content compared to stock 20 and stock 12. However, there were no significant differences in sucrose accumulation between flesh and heart tissue of stock 23 after 8 and 16 weeks of storage, but the highest concentrations of sucrose were recorded in flesh and heart tissue of stock 23 after 16 weeks of storage (27.8 and 27.2 mg g^{-1} DW, respectively). Heart sucrose content of stock 20 was significantly higher when compared to flesh after 8 and 20 weeks of storage. A similar pattern was followed for those tissue samples of stock 12 after 16 and 20 weeks of storage (Fig. 4.4c).

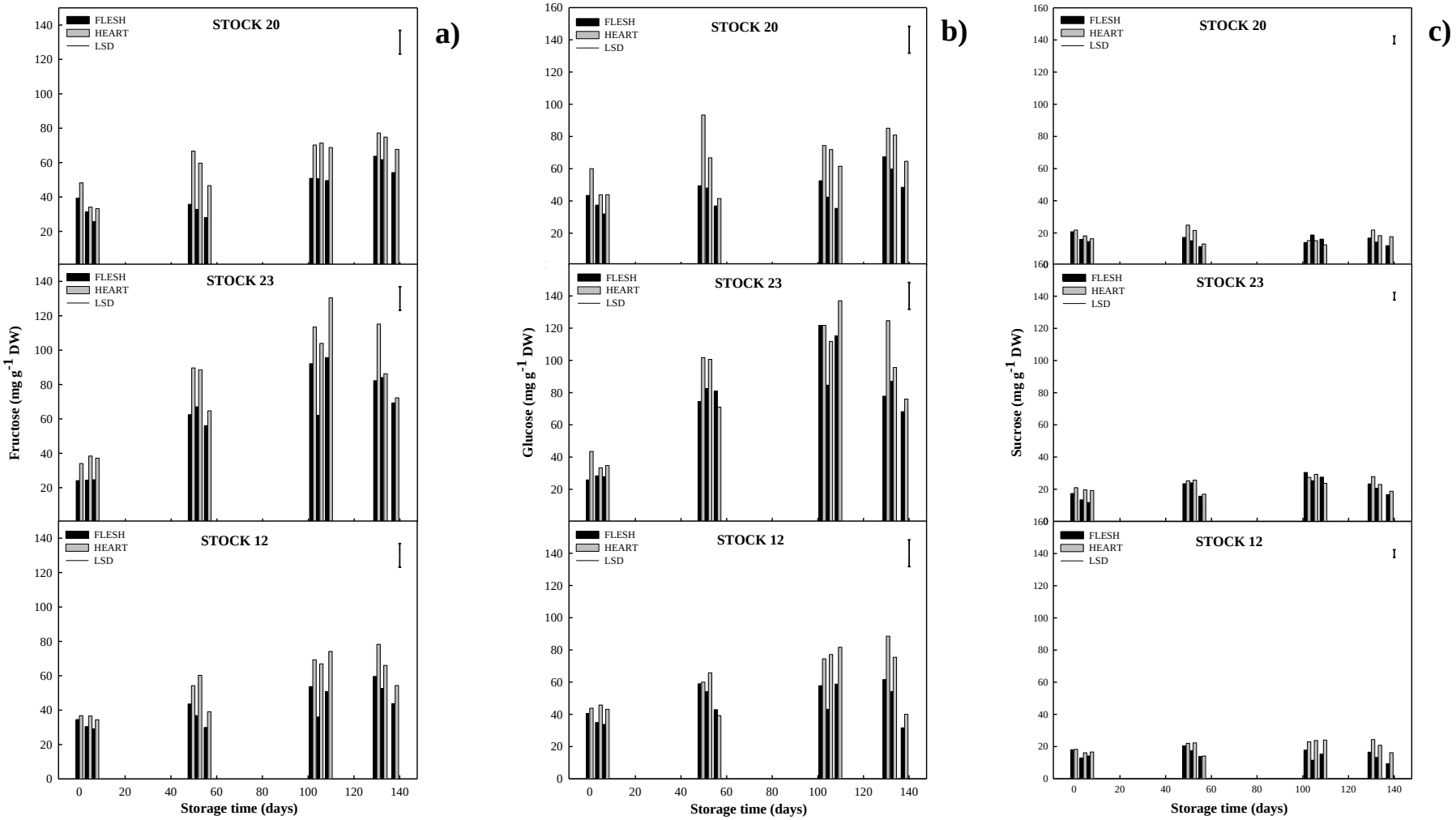


Figure 4.4 Fructose (a), glucose (b) and sucrose (c) concentrations (mg g^{-1} DW) in flesh and heart tissue of potato tubers Maris Piper stock 20 (susceptible to BH), stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) at baseline and after storage in air only at 15^{o}C on sampling days 0, 3 and 7. Tubers were initially stored at 1.5^{o}C for 8, 16 and 20 weeks. Values are means (n= 3). LSDs are shown ($P < 0.05$) (Appendix B; Table 7.1 – 7.3).

4.5.2. Phenolic content analysis

4.5.2.1. Hydroxycinnamic acid derivatives

Results indicated that chlorogenic acid was the major hydroxycinnamic acid accumulated in potato tubers. According to Figure 4.5a, chlorogenic acid tended to be more accumulated in the flesh rather than the heart tissue samples of stock 20 (susceptible to BH) and stock 12 (non-susceptible to BH) but this trend was not followed by stock 23 (susceptible to BH) over storage time. In general, flesh chlorogenic acid was ca. 2-3 times higher than in heart tissue samples of stock 20 and stock 12 while its concentration in flesh and heart of stock 23 was about the same indicating that heart chlorogenic acid was significantly higher in stock 23 compared to those heart samples of stock 20 and stock 12. However, the content of chlorogenic acid varied in stocks on different sampling days. At baseline, flesh chlorogenic acid was ca. 2-3 times significantly higher in stock 12 (199 - 266 μg g^{-1} DW) compared to stock 20 (92 - 116.5 μg g^{-1} DW) and stock 23 (91.2 - 119.4 μg g^{-1} DW) respectively on sampling days 0 and 3, and ca. 6–fold significantly higher than in heart tissue samples (19.9 - 45.2 μg g^{-1} DW). A similar trend was followed by stock 20 where flesh chlorogenic acid was ca. 3 times higher than in heart samples. On the other hand, no significant differences between flesh and heart samples of stock 23 were observed at baseline; however, heart chlorogenic acid content of stock 23 was 2-3 times higher (55 - 76.7 μg g^{-1} DW) compared to those heart samples of stock 20 (23.6 - 49.2 μg g^{-1} DW) and stock 12 (19.9 - 45.2 μg g^{-1} DW) (Fig.4.6a). After 8 and 16 weeks of storage, a similar trend was followed by stock 20 and stock 12 where flesh chlorogenic acid was significantly higher compared to heart samples. However, chlorogenic acid was slightly decreased in those flesh samples of stock 12 and slightly increased in those heart samples of stock 20 after 16 weeks of storage. In terms of stock 23, there were no significant differences in chlorogenic acid content between flesh and

heart samples after 8 and 16 weeks of storage, but it was 2-3 times significantly higher in heart compared with those heart samples of stock 20 and stock 12 at the same period of storage time while there were no significant differences between the flesh samples of all stocks (Fig. 4.5a). After 20 weeks of storage, there was an increase in flesh chlorogenic acid of stock 20 and no significant differences between flesh and heart samples of stock 12 and stock 23. Additionally, there were no significant differences in chlorogenic acid between heart samples of all stocks after 20 weeks of storage (Fig. 4.5a).

Although, chlorogenic acid isomers neo- and crypto-chlorogenic acid are at very low levels in potato tubers, significant differences in both phenolic acids were observed by comparing susceptible (stock 20 and 23) and non-susceptible stocks (stock 12). Neo-chlorogenic acid was always significantly higher in flesh tissue of stock 12 (non-susceptible) when compared to stock 20 and 23 (both susceptible to BH) over storage time (Fig. 4.5b). In general, flesh and heart neo-chlorogenic acid ranged from 0.19 to 4.9 and 0-1.3 μg g^{-1} DW for stock 20, 0.4 - 2.9 and 0.6 - 3 μg g^{-1} DW for stock 23 and 1.3 - 8.6 and 0.1 - 4.2 μg g^{-1} DW for stock 12 respectively. At baseline, neo-chlorogenic acid content in flesh was ca. 3-times increased in stock 12 and significantly decreased in stock 20 while there were no significant differences between those heart samples of all three stocks (Fig. 4.5b). After 8, 16 and 20 weeks of storage there was a significant increase in neo-chlorogenic acid content where it was significantly higher in flesh samples of stock 20 and yet even higher in flesh samples of stock 12. Heart neo-chlorogenic acid content was significantly higher in stock 23 after 8 weeks of storage (2.42 μg g^{-1} DW) while after 16 weeks of storage its concentration (2.41 μg g^{-1} DW) was similar to that of heart samples of stock 12 (2.66 μg g^{-1} DW). After 20 weeks of storage, it was observed that neo-chlorogenic acid concentrations were significantly lower in those heart samples of stock 23 compared to its flesh samples. Furthermore, the content of neo-

chlorogenic acid was always lower in those heart samples of stock 20 over storage time (Fig. 4.5b).

A similar pattern was followed for crypto-chlorogenic acid where it was highly accumulated in flesh tissue of stock 12 (non-susceptible) compared to both susceptible stocks (stocks 20 and 23) over storage time (Fig. 4.5c). Significant differences were shown between both tissue samples of stock 12 where crypto-chlorogenic acid was ca. 5 to 10 times higher in flesh than the heart tissue samples over storage time ranging from 10.57 to 55.42 μg g^{-1} DW and 1.47 - 7.16 μg g^{-1} DW respectively. This trend (crypto higher in flesh compared to heart tissue) was also observed in stock 20 (less susceptible to BH) after baseline where flesh tissue had ca. 2 to 9 greater crypto-chlorogenic acid content ranging from 3.5 to 43.58 μg g^{-1} DW and from 0.89 to 11.37 μg g^{-1} DW respectively. In terms of stock 23, there were no significant differences in crypto-chlorogenic acid among tissue samples over storage time, however, heart crypto-chlorogenic acid content of stock 23 was always significantly higher compared to those heart samples of stock 20 and stock 12 along the storage time ranging from 4.85 to 23.44 μg g^{-1} DW (Fig. 4.5c). No statistical analysis was carried out for the rest of the hydroxycinnamic acids examined (caffeic, *p*-coumaric and ferulic acids) because those compounds were either undetectable or very low in abundance and quantification was unattainable. Both flavonoids rutin and quercetin-3,4-O-diglucoside were identified and quantified in some samples, mainly in flesh tissue, however, due to a high proportion of missing values no statistical analysis was attainable. Therefore, there was zero abundance in isorhmanetin-3-rutinoside and isorhmanetin-3-glucoside.

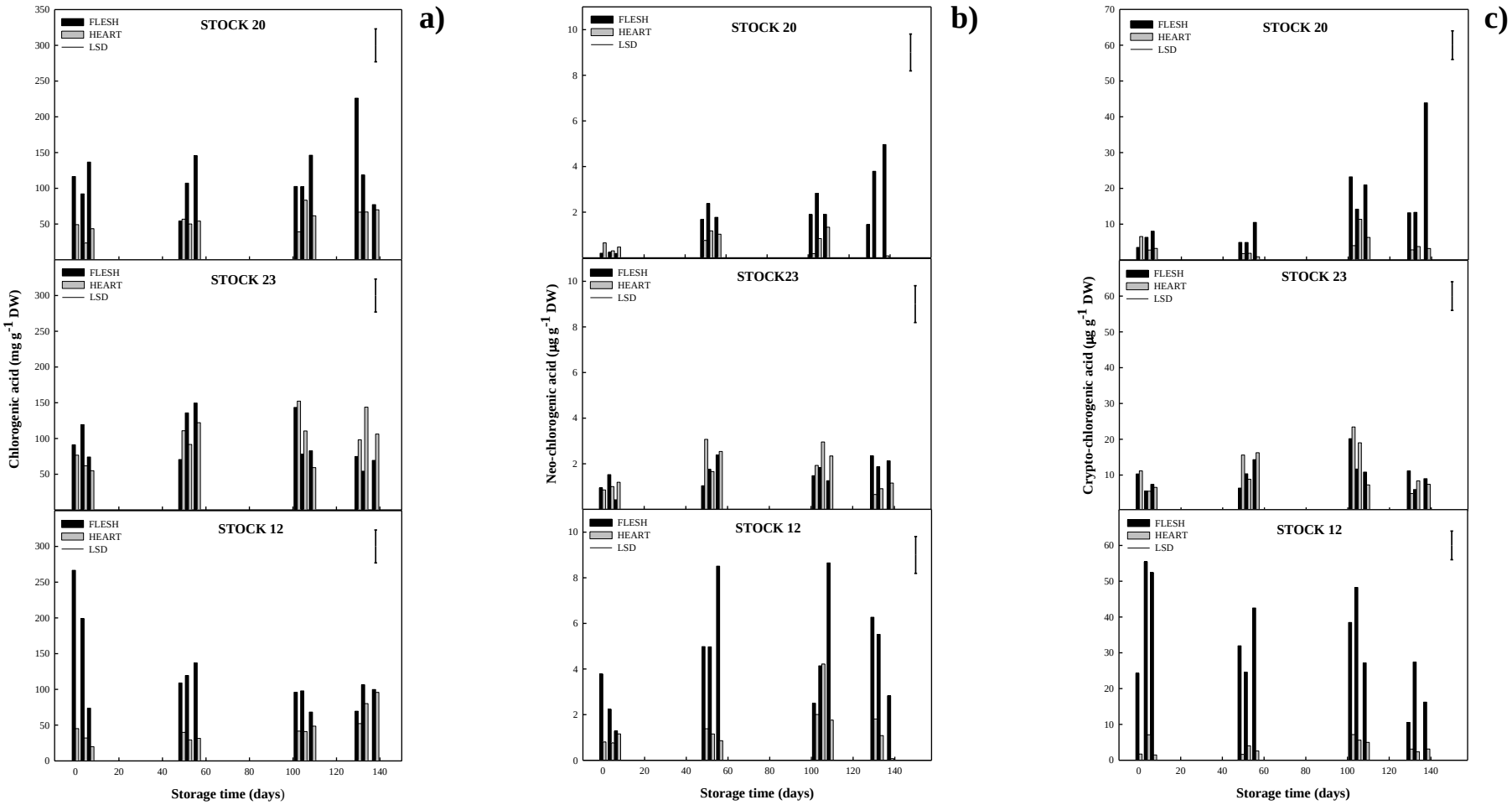


Figure 4.5 Chlorogenic acid (a), neo-chlorogenic acid (b) and crypto-chlorogenic acid (c) concentrations (µg g^{-1} DW) in flesh and heart tissue of potato tubers cv. Maris Piper stock 20 (susceptible to BH), stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) at baseline and after storage in air only at 15^{o}C on sampling days 0, 3 and 7. Tubers were initially stored at 1.5^{o}C for 8, 16 and 20 weeks. Values are means (n= 3). LSDs are shown ($P < 0.05$) (Appendix B; Table 8.1 – 8.3).

According to Figure 4.6a, it was shown that caffeoyl-D-glucose content varied among the three stocks examined [Stock 20 (susceptible to BH), stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) over storage time. At baseline and after 8 weeks of storage, caffeoyl-D-glucose was significantly higher in heart tissue of those samples of stock 23 (0.9-1.3 μg g^{-1} DW) when compared to heart tissue of stock 20 (0.83 - 0.89 μg g^{-1} DW) and stock 12 (0.75 - 0.97 μg g^{-1} DW), but no significant differences were found in flesh tissue of all stocks during that period of storage time. However, after 16 weeks of storage the concentration of caffeoyl-D-glucose was ca. 2 times higher in flesh tissue when compared with heart tissue of those tubers of stock 20 ranging from 0.73 - 0.77 and 0.28 - 0.5 μg g^{-1} DW respectively. Similar trend (caffeoyl-D-glucose higher in flesh samples) was followed by stock 12 (Fig. 4.6a). After 20 weeks of storage, caffeoyl-D-glucose concentration was significantly decreased in flesh and increased in heart tissue of stock 23 ranging from 0.27 - 0.42 and 0.44 - 1.25 μg g^{-1} DW respectively (Fig. 4.6a).

In terms of feruloylquinic acid content, that was found being more accumulated in the flesh tissue samples of stock 20, stock 23 (both susceptible to BH) and stock 12 (non-susceptible) ranging from 4.59 - 21.6, 5.66 - 12.08 and 1.33 - 15.75 μg g^{-1} DW respectively compared to those heart tissue samples of all stocks (Fig 4.6b). After baseline, flesh feruloylquinic acid content was ca. 2 times higher when compared to heart samples of all stocks analysed and yet even higher in flesh samples of stock 20 (19.74 - 216 μg g^{-1} DW). There were no differences of feruloylquinic acid content in both susceptible stocks (stock 20 and stock 23) after 8 and 16 weeks of storage. Feruloylquinic acid concentrations in heart tissue of stock 12 were significantly lower on sampling day 3 after 8 weeks of storage and also significantly lower in flesh tissue on sampling day 7 after 16 weeks of storage respectively (Fig. 4.6b). After 16 weeks of storage, it was observed that heart feruloylquinic acid (15.43 μg g^{-1} DW) was significantly increased compared to flesh (9.38 μg g^{-1} DW)

samples of stock 23. Furthermore, after 20 weeks of storage, feruloylquinic acid content in flesh samples of stock 20 and stock 23 (both susceptible to BH) was significantly higher on sampling day 0 when compared to sampling day 7. At the same period of storage time, it was shown that heart feruloylquinic acid content of stock 23 decreased on sampling day 7. However, on sampling days 0 and 3, flesh feruloylquinic acid of stock 12 (non-susceptible to BH) was significantly higher (ca. 2 times) than in heart tissue (Fig. 4.6b).

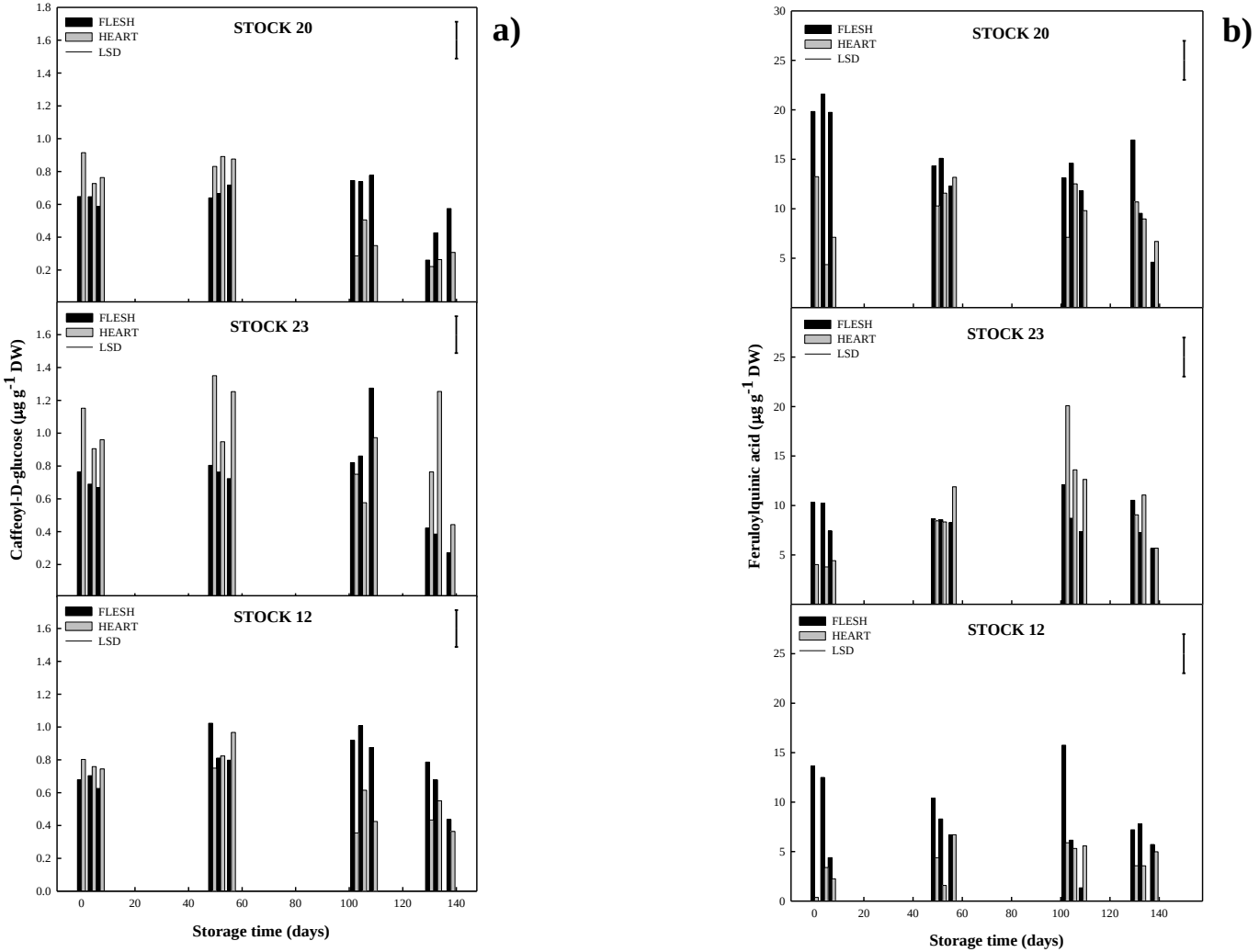


Figure 4.6 Caffeoyl-D-glucose (a) and feruloylquinic acid (b) concentrations (µg g^{-1} DW) in flesh and heart tissue of potato tubers cv. Maris Piper stock 20 (susceptible to BH), stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) at baseline and after storage in air only at 15^{o}C on sampling days 0, 3 and 7. Tubers were initially stored at 1.5^{o}C for 8, 16 and 20 weeks. Values are means (n= 3). LSDs are shown (P < 0.05) (Appendix B; Table 8.4 – 8.5).

4.5.2.2. Aromatic amino acids

A variation in content of those three amino acids quantified (tyrosine, phenylalanine and tryptophan) was shown along storage time. Tyrosine content was significantly higher in stock 20 (susceptible to BH) (mean = 0.8 µg g^{-1} DW) and lower in stock 12 (non-susceptible to BH) (mean = 0.4 µg g^{-1} DW) at baseline. After 8 weeks of storage, flesh tyrosine was significantly decreased in stock 20 (0.27 µg g^{-1} DW) and stock 23 (susceptible to BH) (0.26 µg g^{-1} DW), but heart tyrosine of stock 20 was ca. 2 times higher (0.54 µg g^{-1} DW) (Fig. 4.7a). Furthermore, heart tyrosine content was ca. 3 times higher in stock 20 (1.74 µg g^{-1} DW) after 16 weeks of storage and flesh tyrosine was also higher (0.94 µg g^{-1} DW). A similar trend for tyrosine content in heart samples of stock 23 was followed after 16 and 20 weeks of storage (1.42 and 1.3 µg g^{-1} DW respectively). There were no really differences in flesh tyrosine concentrations in stock 12 overs storage time (Fig. 4.7a).

Phenylalanine content was significantly lower in stock 12 (11.4 µg g^{-1} DW) compared to stock 20 (23.7 µg g^{-1} DW) and stock 23 (21.9 µg g^{-1} DW) at baseline and yet there were no significant differences between the latter stocks (Fig. 4.7b). After 8 weeks of storage, flesh phenylalanine was significantly decreased in stock 20 (11.9 µg g^{-1} DW) and stock 23 (9.7 µg g^{-1} DW) but there were no differences compared to stock 12 (8.53 µg g^{-1} DW) while heart phenylalanine significantly increased in stock 20 and stock 23 (22.67 and 21.2 µg g^{-1} DW respectively). Flesh phenylalanine concentration was increased in all stocks after 16 weeks of storage but there were no significant differences between the stocks. However, heart phenylalanine was significantly higher in heart compared to flesh samples of stock 23 after 16 weeks of storage (30.8 and 21.3 µg g^{-1} DW respectively). After 20 weeks phenylalanine content varied among the stocks (Fig. 4.7b).

Generally, tryptophan content, was significantly lower in stock 12 (mean = 72.3 µg g^{-1} DW) compared to stock 20 and stock 23 (means = 118.2 and 121 µg g^{-1} DW respectively) and yet lower in heart compared to flesh samples with a mean of 95 and 111 µg g^{-1} DW respectively. Stock 12 (non-susceptible to BH) had the lowest amount in tryptophan (ca. 2- to 3-fold lower) at baseline and after 16 and 20 weeks of storage when compared to both susceptible stocks (stock 20 and 23) (Fig. 4.7c).

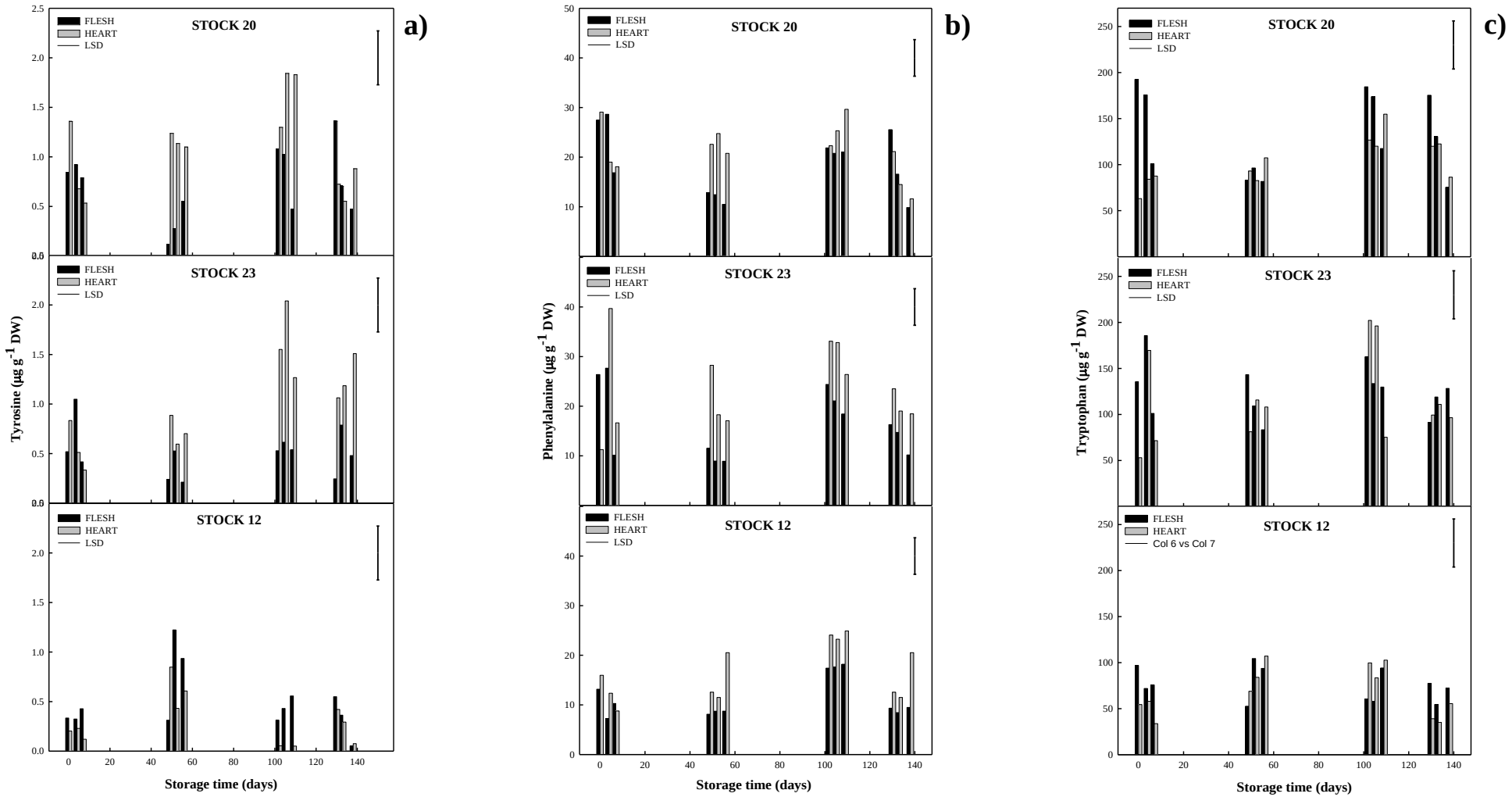


Figure 4.7 Tyrosine, phenylalanine and tryptophan concentrations (µg g^{-1} DW) in flesh and heart tissue of potato tubers cv. Maris Piper stock 20 (susceptible to BH), stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) at baseline and after storage in air only at 15^{o}C on sampling days 0, 3 and 7. Tubers were initially stored at 1.5^{o}C 8, 16 and 20 weeks. Values are means (n= 3). LSDs are shown ($P < 0.05$) (Appendix B; Table 8.6 – 8.8).

4.5.3. Multivariate analysis

Principal Component Analysis (PCA) was used as an unsupervised multivariate technique finding differences between sample groups converting a number of possible correlated variables into a smaller number of uncorrelated variables called principal components or clusters. Three sugar variables (fructose, glucose and sucrose) combined with eight phenolic compounds (chlorogenic acid, neo-chlorogenic acid, crypto chlorogenic acid, caffeoyl-D-glucose, feruloylquinic acid, tyrosine, phenylalanine and tryptophan) were considered as analytical data for PCA. PCA was performed for a better visualization of sugar and phenolic accumulation in flesh and heart tissue samples of all three stocks analysed [stock 20 (susceptible to BH), stock23 (susceptible to BH) and stock 12 (non-susceptible to BH)] at each sampling point separately. All three stocks had been stored in air only at 15^{o}C, sampled on three days (*viz.* day 0, 3 and 7) per four weeks and initially stored at 1.5^{o}C for 8, 12, 16 and 20 weeks of storage (sampling points). It is worth reminding the reader that there was no cold initial storage temperature at baseline. In addition, general PCA biplots of flesh and heart samples were designed over storage time (Fig. 4.12a and b respectively). In all PCA biplots flesh and heart tissue samples were labelled according to stock [1= stock 20 (susceptible to BH), 2= stock 23 susceptible to BH) and 3= stock 12 (non-susceptible to BH)] corresponded with tissue discoloration [*viz.* dark brown to black (BH), brown centre (BC), brown centre light (BCL) and pith] BH-like discoloration was only observed in stock 23 samples. Control samples of stock 20 and stock 23 were further labelled as 'C' meaning control= no discoloration. It should be noted that those flesh samples of both stocks 20 and 23 labelled according to tissue discoloration (i.e. 1PITH or 2BC) did not show any tissue discoloration, but labelling was carried out in that way in order to clarify the affected tuber from which they derived and to show any possible similarities or differences in sugar and phenolic content compared to control samples. Samples of stock 12 considered as non-

susceptible showing no tissue discoloration were labelled as 3. At baseline there were no indications of tissue discoloration, so labelling was carried out according to stock only.

At baseline before transferring the tubers at cold storage temperature, the PCA on flesh tissue samples showed a separation of all three stocks analysed on PC1 (captured 55.50% of the variance) and PC2 (captured 38.35% of the variance) capturing, in total, almost 94% of the variance (Fig. 4.8a). A clear separation for those flesh samples of stock 12 was observed which according to the biplot corresponded with high chlorogenic acid and its isomers (neo- and crypto- chlorogenic acid) (Fig. 4.8a). Flesh samples of stock 20 tended to have more sucrose and feruloylquinic acid content while an accumulation of the three amino acids was also observed. No clear separation for flesh samples of stock 23 was observed. The PCA on heart tissue samples was shown on PC1 (captured 71.10% of the variance) and PC2 (captured 20.95% of the variance) capturing almost 92% of the variance in total (Fig 4.8b). Chlorogenic acid with its isomers and caffeoyl-D-glucose seemed to be important variables for separating stock 23 and reducing sugars for differentiating stock 20 from the other stocks (Fig. 4.8b).

The PCA on the flesh tissue data after 8 weeks of storage at 1.5^{o}C showed a clear separation of all three stocks on PC1 (captured 57.94% of the variance) and PC2 (captured 25.17% of the variance) capturing, in total, almost 83% of the variance (Fig. 4.9a). Again, stock 12 was mainly grouped according to chlorogenic acid and its isomers and caffeoyl-D-glucose as well. Feruloylquinic acid and phenylalanine was the main variable for differentiating stock 20 from the other stocks analysed According to the biplot, it was observed that sugars started to be more accumulated in flesh of stock 23 and also tryptophan seemed to be less important variable for separating stock 23 from the other stocks (Fig. 4.9a). After 8 weeks of storage, indications of pith discolorations were initially observed in heart samples of stock 20 and stock 23. The PCA on the heart tissue data after 8 weeks of storage at 1.5^{o}C was shown on PC1 (captured 55.91% of the variance) and PC2 (captured 26.88% of the

variance) capturing, in total, almost 83% of the variance (Fig. 4.9b). A good grouping was only observed for those control heart samples stock 23 corresponded with higher chlorogenic acid and isomers contents, phenylalanine and caffeoyl-D-glucose and also high sugar content. Also, sugar content tended to be more accumulated in those heart samples of stock 23 showing pith discoloration. A clear separation for those heart samples from stock 20 and stock 12 was not observed (Fig. 4.9b).

After 16 weeks of storage at low initial storage temperature (1.5^{o}C), sugar and phenolic content tended to accumulate more in stock 23 (susceptible to BH). According to Figure 4.10a, the PCA on flesh data was shown on PC1 (captured 59.21% of the variance) and PC2 (captured 26.82% of the variance) capturing, in total, almost 86% of the variance. The most important variables for separating flesh samples of stock 23 were sugars, tryptophan and tyrosine and the less important were phenylalanine, feruloylquinic acid and caffeoyl-D-glucose (Fig. 4.10a). Once again, chlorogenic acid and its isomers were the main variables for differentiating those flesh samples of stock 12 (non-susceptible to BH). No clear separation for stock 20 was distinguished. According to Figure 4.10b, the PCA on heart tissue data was shown on PC1 (captured 53.27% of the variance) and PC2 (captured 33.63% of the variance) capturing, in total, almost 87% of the variance. In general, stock 23 was differentiated by all the variables examined. Most of the discoloured heart samples of stock 23 tended to be grouped according to sugars (mainly sucrose), caffeoyl-D-glucose and neo-chlorogenic acid while chlorogenic acid, crypto-chlorogenic acid, reducing sugars, amino acids and feruloylquinic acid were those important variables for separating those control heart samples of stock 23. Control heart samples of stock 20 tended to be grouped according to tyrosine and tryptophan (Fig. 4.10b).

At the last sampling point (20 weeks of storage), the PCA on flesh tissue data showed a separation of stocks on PC1 (captured 58.38% of the variance) and PC2 (captured 31.28% of

the variance) capturing, in total, almost 90% of the variance (Fig. 4.11a). Sugars were the most important variables and tyrosine less important for differentiating stock 23 (susceptible to BH) from stock 20 (susceptible to BH) and stock 12 (non-susceptible to BH). Stock 12 was mainly grouped according to caffeoyl-D-glucose, neo- and crypto-chlorogenic acids. Flesh samples of stock 20 tended to have higher concentration of feruloylquinic acid and phenylalanine (Fig. 4.11a). According to Figure 4.11b, the PCA on heart data was shown on PC1 (captured 55.03% of the variance) and PC2 (captured 30.83% of the variance) capturing, in total, almost 86% of the variance (Fig. 4.11b). Control heart samples of stock 23 were mainly separated according to phenylalanine, chlorogenic acid and its isomers while discoloured heart samples were mainly grouped according to sugars and tryptophan and less to caffeoyl-D-glucose, feruloylquinic acid and tyrosine (Fig. 4.11b).

A general PCA of flesh data over storage time showed a stock separation on PC1 (captured 51.62% of the variance) and PC2 (captured 33.21% of the variance) capturing, in total, almost 85% of the variance (Figure 4.12a). According to the PCA biplot, sugars were the main variables for separating stock 23 (susceptible to BH) and chlorogenic with its isomers for stock 12 (non-susceptible to BH) (Fig. 4.12a). Furthermore, a general PCA was performed on heart data over storage time which was shown on PC1 (captured 44.57% of the variance) and PC2 (captured 37.94% of the variance) capturing, in total, almost 82.5% of the variance (Fig. 4.12b). Over storage time, control heart samples of stock 23 were mainly clustered according to chlorogenic acid and its isomers, but a few control and discoloured samples tended to be more accumulated according to sugar content and amino acids (Fig. 4.12b).

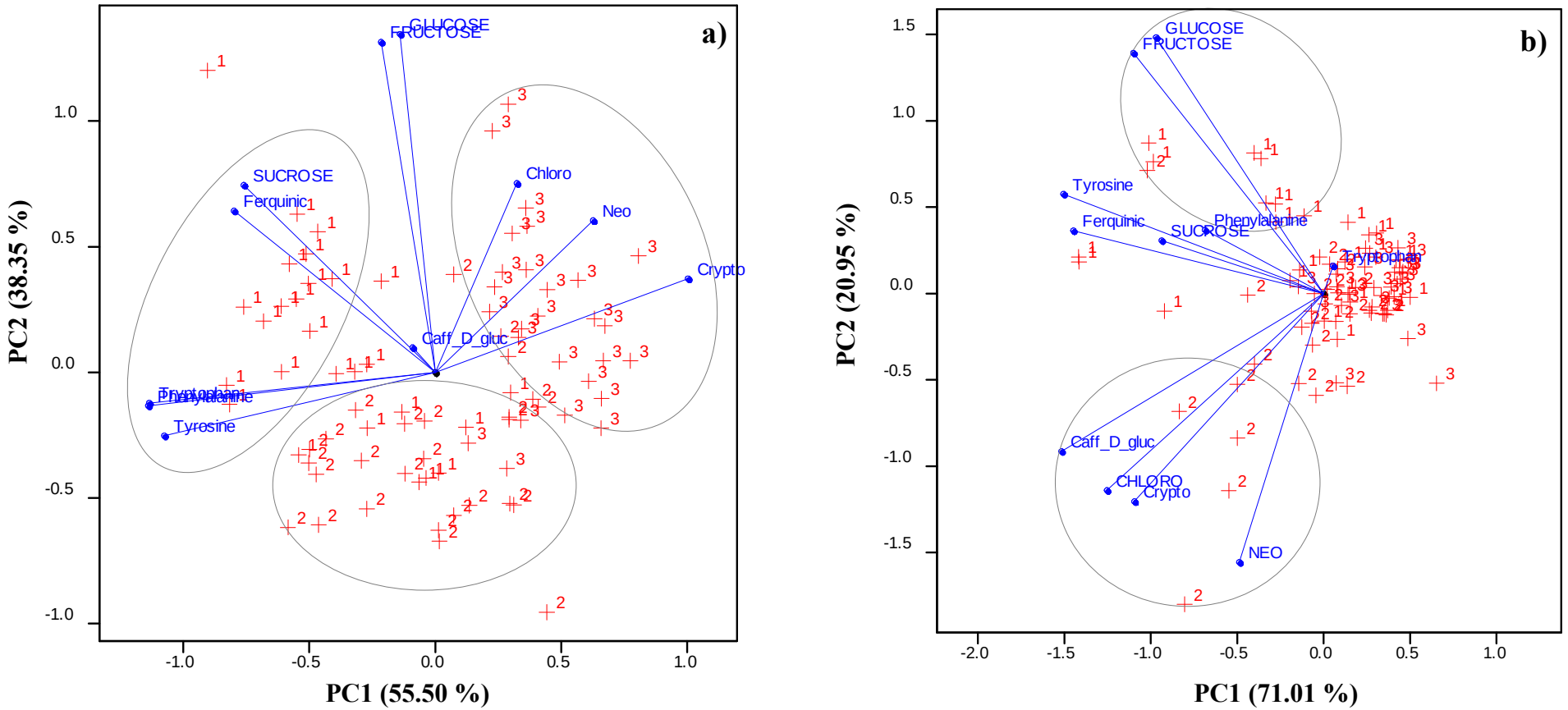


Figure 4.8 PCA biplot of flesh (a) and heart (b) tuber tissue labelled by stock [1= stock 20 (susceptible to BH), 2= stock 23 (susceptible to BH) and 3= stock 12 (non-susceptible to BH)]. Tubers were stored at 15^{o}C in air on sampling days 0, 3 and 7 at baseline. There was no initial storage temperature at baseline.

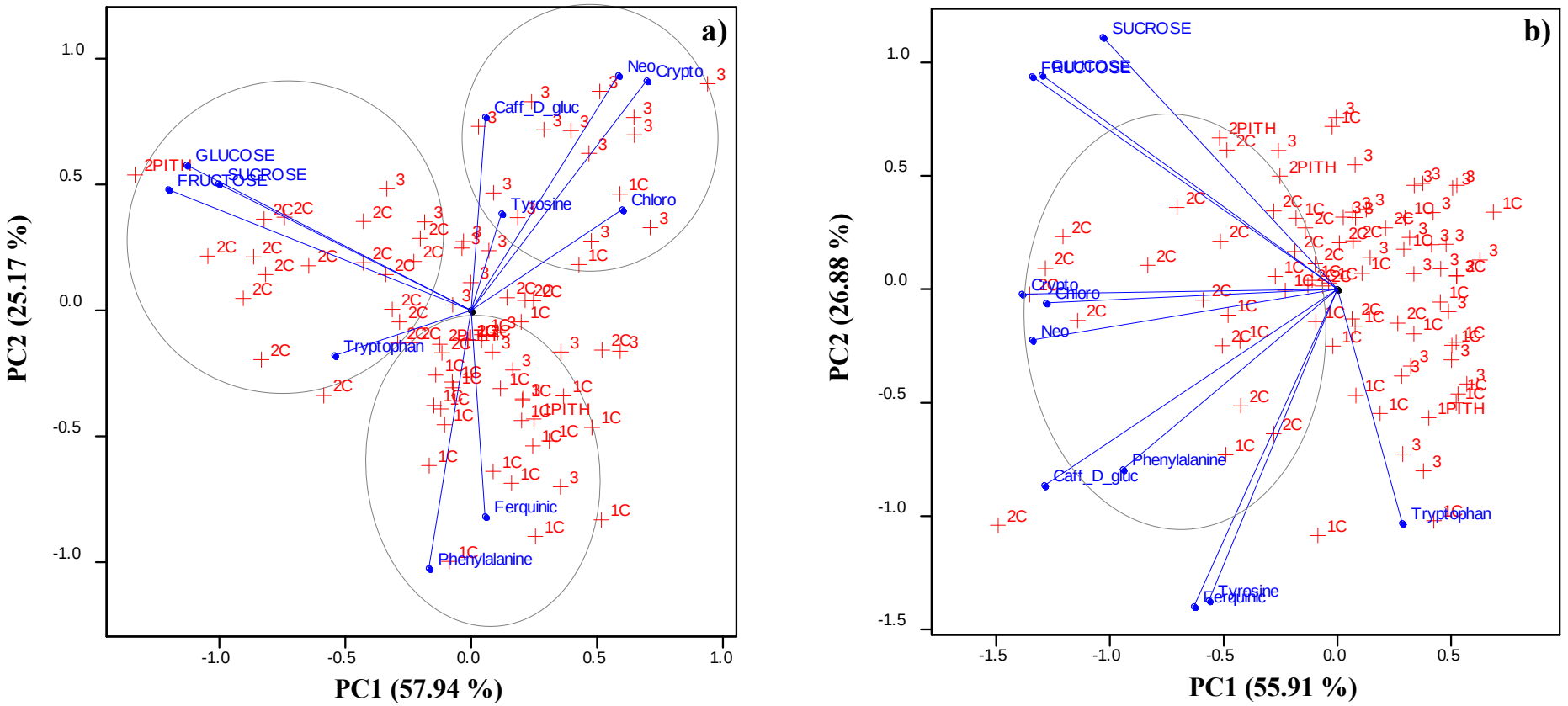


Figure 4.9 PCA biplot of flesh (a) and heart (b) tuber tissue labelled by corresponding to tissue discoloration [1= stock 20 (susceptible to BH), 2= stock 23 (susceptible to BH) and 3= stock 12 (non-susceptible to BH); C= non-affected (control) samples of stock 20 and stock 23. PITH, brown centre light (BCL), brown centre (BC), dark brown to black (BH) = affected samples of stock 20 and stock 23. Tubers were stored at 15^{o}C in air on day 49, 52 and 56 (sampling days 0, 3 and 7 respectively), and initially stored at 1.5^{o}C for 8 weeks.

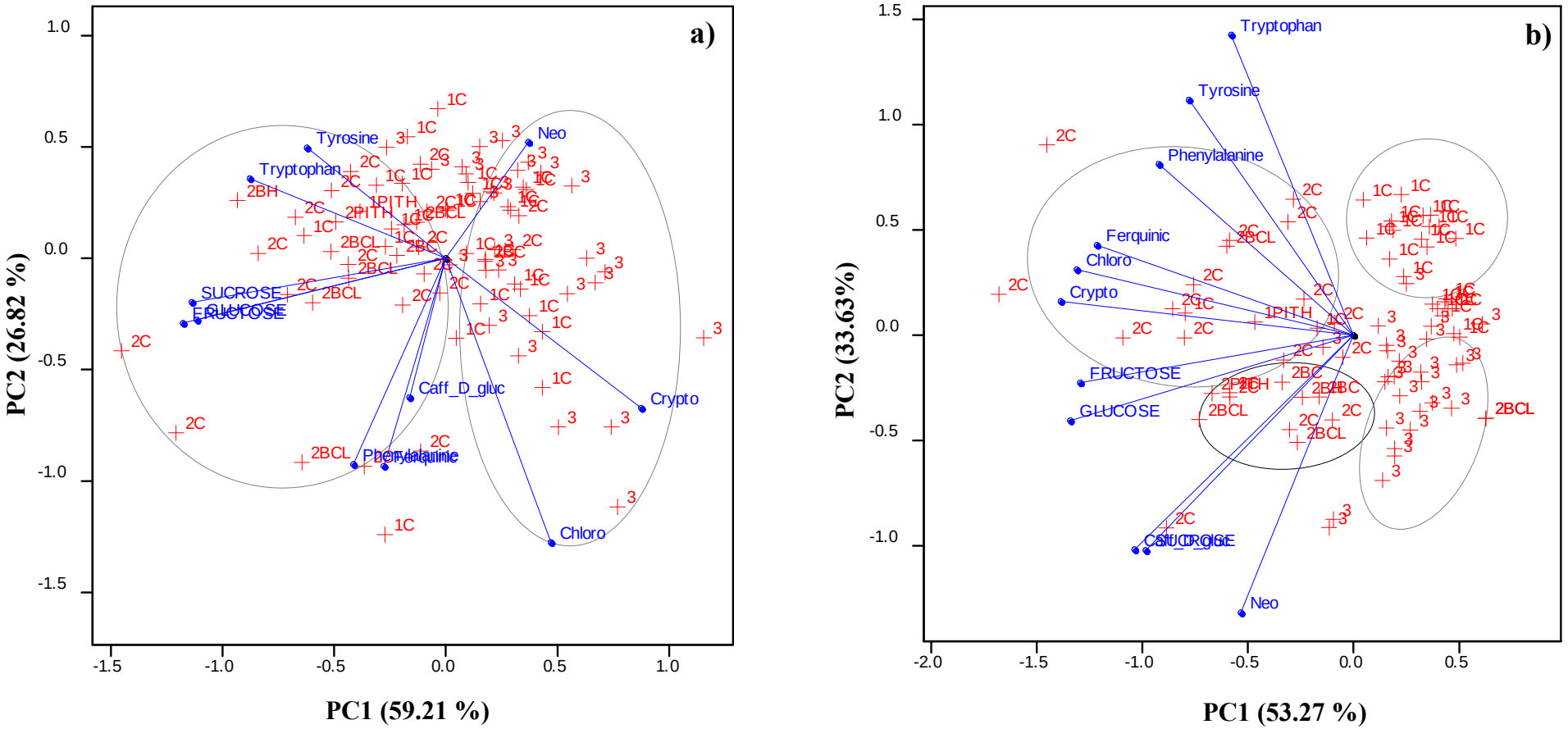


Figure 4.10 PCA biplot of flesh (a) and heart (b) tuber tissue labelled by corresponding to tissue discoloration [1= stock 20 (susceptible to BH), 2= stock 23 (susceptible to BH) and 3= stock 12 (non-susceptible to BH); C= non-affected (control) samples of stock 20 and stock 23. PITH, brown centre light (BCL), brown centre (BC), dark brown to black (BH) = affected samples of stock 20 and stock 23. Tubers were stored at 15^{o}C in air on day 102, 105 and 109 (sampling days 0, 3 and 7 respectively), and initially stored at 1.5^{o}C for 16 weeks.

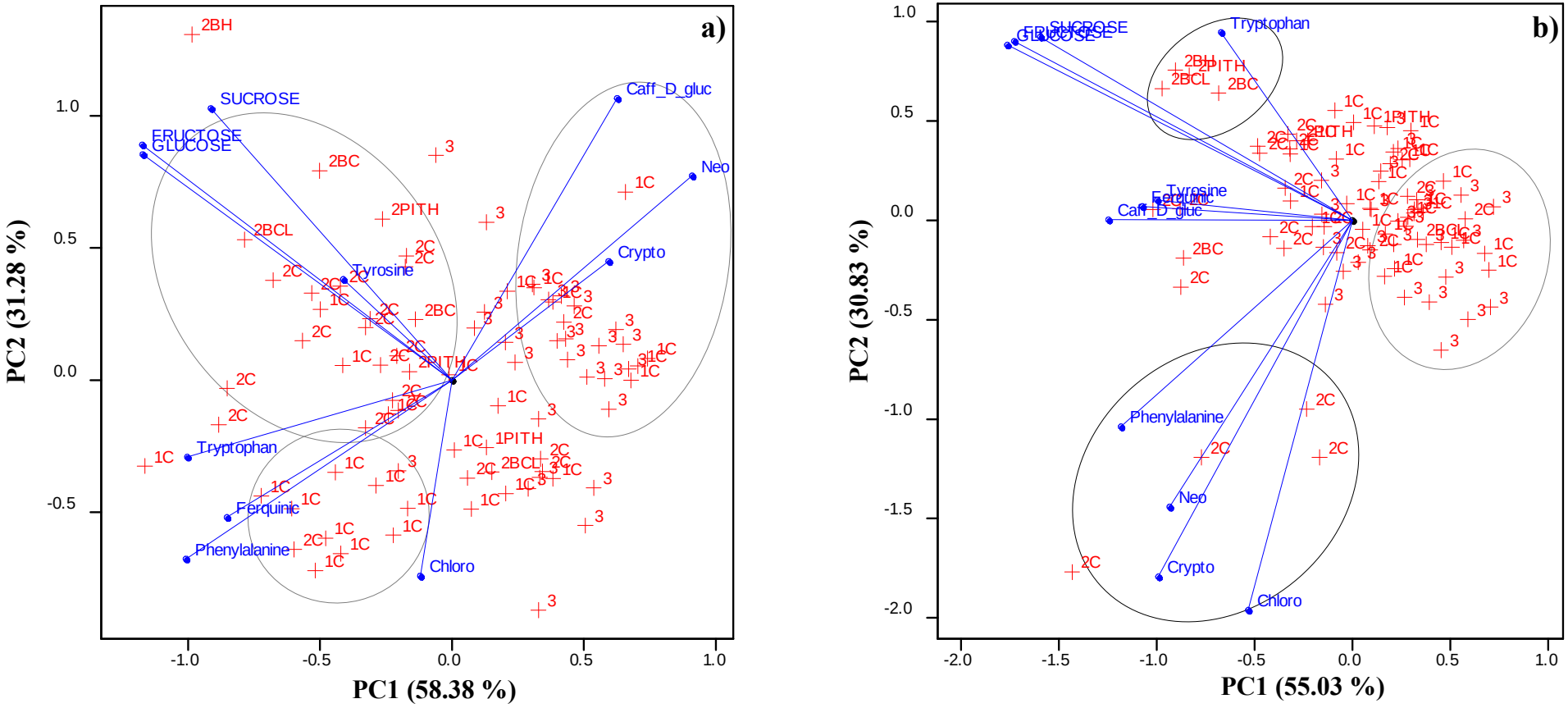


Figure 4.11 PCA biplot of flesh (a) and heart (b) tuber tissue labelled by corresponding to tissue discoloration [1= stock 20 (susceptible to BH), 2= stock 23 (susceptible to BH) and 3= stock 12 (non-susceptible to BH); C= non-affected (control) samples of stock 20 and stock 23. PITH, brown centre light (BCL), brown centre (BC), dark brown to black (BH) = affected samples of stock 20 and stock 23. Tubers were stored at 15^{o}C in air on day 130, 133 and 138 (sampling days 0, 3 and 7 respectively), and initially stored at 1.5^{o}C for 20 weeks.

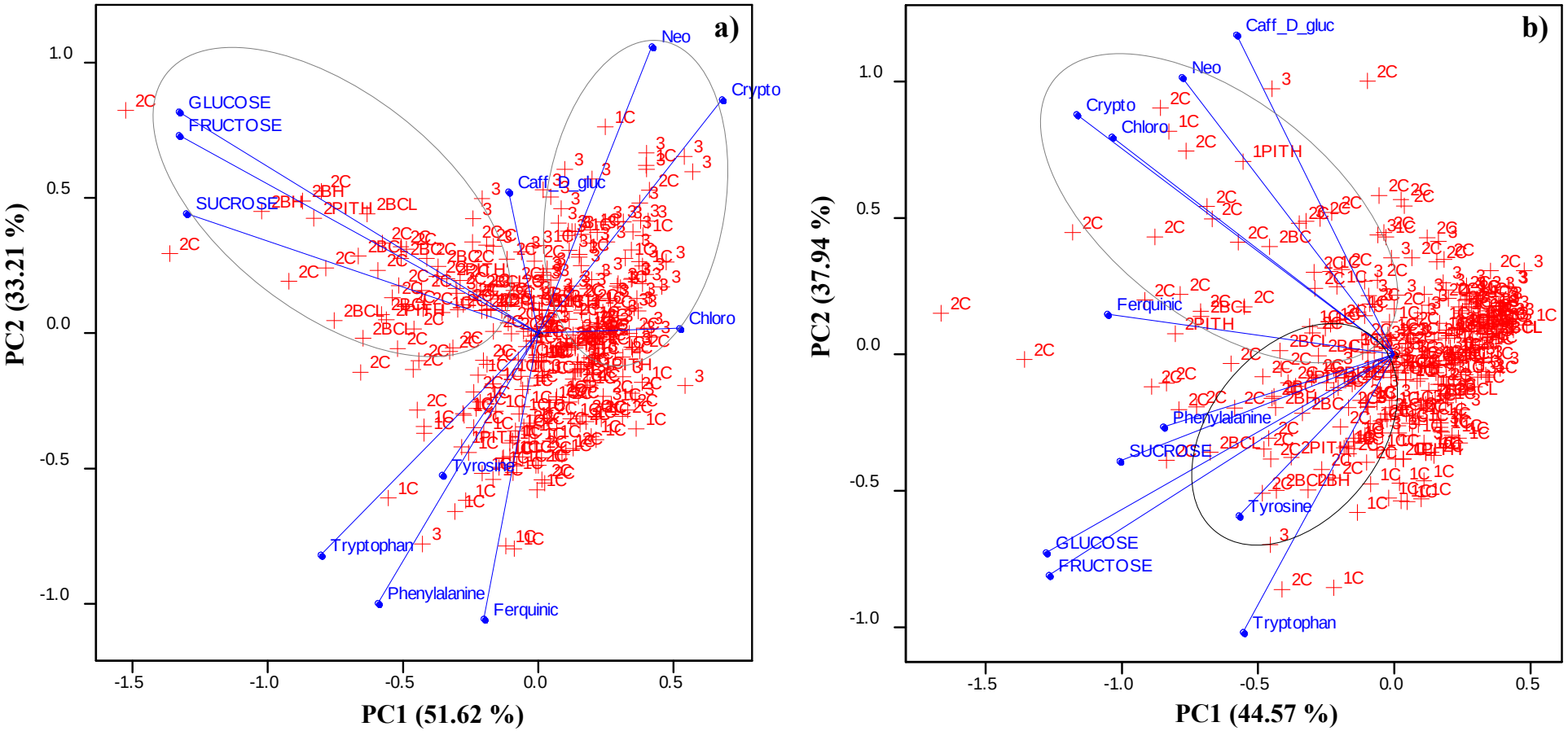


Figure 4.12 General PCA biplot of flesh (a) and heart (b) tuber tissue time labelled by corresponding to tissue discoloration [1= stock 20 (susceptible to BH), 2= stock 23 (susceptible to BH) and 3= stock 12 (non-susceptible to BH); C= non-affected (control) samples of stock 20 and stock 23. PITH, brown centre light (BCL), brown centre (BC), dark brown to black (BH) = affected samples of stock 20 and stock 23. Biplots formed over storage time.

4.6. Discussion

4.6.1. Effect of storage temperature and shelf-life conditions on the respiration rate of potato stocks with different susceptibility to BH

Shelf-life of potatoes as affected by previous conditions and storage that in turn are related with temperature and atmospheric conditions. Respiration rate is considered a key indicator of the potato physiological activity. Fresh potatoes are commercially stored at low temperatures (3-4^{o}C) in order to extend their storage life providing year round availability and thus creating desirable conditions for better shelf-life (Gast, 1991; Kleinkopf, 1995; Mathooko, 1996; Thompson, 1996; Fennir *et al.*, 2003; Nourian *et al.*, 2003). In this study, potato tuber stocks with different susceptibility to BH were stored in air (21% O_2) or CA (10% CO_2 and 18-19% O_2), sampled at 15^{o}C and initially stored at 1.5 or 3^{o}C with the former cold temperature selected as more challenging for the tuber respiration rate and yet greater potential of the BH development. According to the results, it seems that when tubers were transferred from the coldest initial storage temperature (1.5^{o}C) to 15^{o}C in either air or CA storage (10% CO_2) and stored for a further 3 or 7 days there was a greater effect on the tuber respiration rates compared to those that had initially been stored at 3^{o}C. That was evident after 8 weeks of storage at cold temperature, as during baseline assessment there was no initial storage temperature. Once initial storage temperature was applied, then that temperature change effect from cold to warmer storage temperature influenced in a similar way the respiration rate of all stocks analysed. Similar trends in respiration rate were observed by Craft (1963) studying the influence of initial storage temperature on potato respiration rate. After initial storage at 0^{o}C or 12.8^{o}C for 2 months, respiration rate was recorded at 25^{o}C for 6 days with a 12 – 16 h prior warming up. The CO_2 production and O_2

uptake were higher after initial storage at 0^{o}C than at 12.8^{o}C where an increase in respiration rate was shown from day 0 to day 1 and then gradually declined reaching those respiration rates recorded by tubers initially held at 12.8^{o}C that remained stable after 6 days. Craft (1963) called this increase a 'respiration burst' and concluded that is actual respiration rate. Changes in respiration rate caused by temperature fluctuations have previously been reported by Schippers (1977a) where it was said that initial storage at low temperatures stimulated the respiration rate recorded at warmer temperatures after, but there are also a lot other factors affecting those changes in respiration rate such as storage time, potato cultivar, sprouting, tuber size etc. The author also stated that respiration rate will eventually stabilize after fluctuations in storage temperature, but this requires some time to occur (Schippers, 1977a). Herein, indications of dormancy break and sprouting were expected due to storage at 15^{o}C and observed on each sampling days 3 and 7 initiating after storage in either air or CA and initial storage at 1.5 or 3^{o}C for 8 to 20 weeks, thus it might have an effect on increasing the respiration rate (Schippers, 1977a; Dwelle and Stallknecht, 1978; Fennir *et al.*, 2002), but the fact that CO_2 rates were lower and sprouting more evident after initial storage at 3^{o}C, a direct relation between respiration and sprouting cannot be concluded. Similarly, sugar accumulation at very low temperature may be correlated with increased respiration rate (Dwelle and Stallknecht, 1979; Workman *et al.*, 1979; Sherman and Ewing, 1982; Duplessis *et al.*, 1996; Zhou and Solomos, 1998); however, sugar content quantification was only carried out for those tubes stored in air only and initially stored at 1.5^{o}C where BH incidence was more evident.

It is well known that higher temperatures effectively increase the rate of respiration of tubers as do very cold storage temperatures (< 5^{o}C) (Dwelle ans Stallknecht, 1978; Workman *et al.*, 1979; Duplessis *et al.*, 1996; Zhou and Solomos, 1998; Kumar, 2011). BH disorder

does not occur under optimal storage temperatures when O_2 is efficiently supplied, is induced at extremely high or low temperatures demanding higher O_2 levels and development may also occur at around 5^{o}C but longer time is required (Bartholomew, 1916; Davis, 1928; Link *et al.*, 1932; O'Brien and Rich, 1976; Smith, 1978; Hooker, 1981; Wale *et al.*, 2008; Kumar Chaurasia, 2009; Voss, UC Davis; Robinson and Secor, NDSU). However, in this present study a similar pattern distinguishing significant differences in respiration rate between potato stock susceptibility to BH was not clearly shown over storage time except during baseline measurements. There were some significant differences in CO_2 production between the stocks analysed but these were not consistent across sampling days during storage. That might happen due to low BH incidence indicated at 15^{o}C or because respiration rates were recorded in a jar containing both affected and non-affected tubers in the case of the susceptible stocks (20 and 23) and also three tubers were placed in the jar (see Chapter 3, session 3.4). Moreover, it could be said that O_2 was not really depleted in those chambers used during air storage where O_2 concentrations were at 21% and under 10% CO_2 with 18-19% O_2. After storage in air at 15^{o}C and initial storage at 1.5^{o}C respiration rate ranged from ca. 3 - 4, 7 - 8 and 6 - 7 ml CO_2 kg^{-1} h^{-1} and initial temperature 3^{o}C 2.4 - 3, 3.8 - 4.5 and 3.6 - 5 ml CO_2 Kg^{-1} h^{-1} on sampling days 0, 3 and 7 respectively, increasing 2-3 fold from day 0 to day 3 and then slightly decreasing from day 3 to day 7. Voss (UC Davis) suggested that respiration rate in mature tubers may range between 5 – 12 ml CO_2 kg^{-1} h^{-1} at 15^{o}C and Fennir *et al.* (2002) reported that CO_2 rates of potato tubers cv. Chieftain ranged from 6 – 7.6 ml CO_2 kg^{-1} h^{-1} after 29 days at 15^{o}C. Furthermore, that increase in respiration rate observed after storage at 10% CO_2 was more likely due to the CO_2 absorption as a similar trend in respiration rate was followed by all stocks analysed. Yet, BH incidence did not differ between different storage conditions. So, after storage in CA at 15^{o}C and initial storage at 1.5 or 3^{o}C respiration rate ranged from ca. 12.3 – 15.4 and 9.4 – 12.6 ml CO_2 kg^{-1} h^{-1} respectively

on sampling days 3 and 10.7 – 15.4 ml CO_2 kg^{-1} h^{-1} and 9.4 – 13.4 ml CO_2 kg^{-1} h^{-1} respectively on sampling days 7 over storage time. In their early work, Perez-Trejo *et al.* (1981) also examined the influence of high CO_2 concentrations on respiration rate of potato tubers cv. Norchip revealing that at 10% CO_2 respiration rate was stimulated and ranged from more than 10 and less than 20 ml CO_2 kg^{-1} h^{-1} due to CO_2 absorption whereas respiration rate recorded in air (21% O_2) was ca. 5 ml CO_2 kg^{-1} h^{-1} at 23^{o}C.

4.6.2. The effect of storage temperature and shelf-life conditions on the incidence of BH of potato stocks with different susceptibility to BH

Blackheart disorder (BH) is a particular problem for the potato packing industry and customer complaints arising around the end of winter peaking in spring time. However, due to similar tissue discolorations occurred in the tubers, sometimes those discolorations might confuse and mislead differentiating the disorder. That happens due to the overlapping with other physiological internal disorders induced in potato tubers showing similar symptoms with BH, severity depended (i.e. brown centre, BC; internal necrosis; hollow heart, HH etc.). It has been proposed that BC is the precursor for HH development and possibly the initial step for BH induction (Reeve, 1968) but this statement is misleading as almost all internal physiological disorders induced in potato tubers share a common feature; that of brown tissue discoloration and yet cell necrosis in some cases (Dinkel, 1963; Van Denburgh *et al.*, 1980, 1986; Bussan, 2007; Sowokinos, 2007; Wale *et al.*, 2008). Furthermore, it has been suggested that physiological disorders and tissue discolorations may be initiated at pre-harvest conditions such as growth conditions, soil temperatures and types, water logging (flooding), calcium deficiency (Link *et al.*, 1932; O'Brien and Rich, 1976; Bussan, 2007; Wale *et al.*, 2008; Palta, 2010; Zommic *et al.*, 2013). It was previously stated that cool growing

conditions at 10 - 15^{o}C soil temperatures BC may be expressed in growing potatoes cv. Russet Burbank (Van Denburgh *et al.*, 1980, 1986). In his recent work on translucency (physiological disorder of potatoes), Zommic *et al.* (2013) reported that warm soil temperatures during potato bulking resulted in severe BH incidence 270 days after harvest after storage at 9^{o}C. In this study, less and more intense brown tissue discolorations localized in the central pith part of the tuber were mostly indicated in both susceptible to BH stocks (stock 20 and stock 23) starting in December 2012 after 8 weeks of storage at very low temperature (1.5 or 3^{o}C) peaking after a few months during springtime. However, the total percentage of BH incidence was quite low (< 10%) corresponding to the total number of tubers used in this experiment. Stock 23 had significantly (ca. 3-fold) greater BH incidence compared to stock 20 and yet BH-like symptoms namely a dark brown to blackish discoloration were indicated in just three tubers of stock 23 after 16 to 20 weeks of storage at 1.5^{o}C. Furthermore, no indications of discoloration were observed at baseline where no initial storage temperature occurred (November 2011) which probably suggests that the initial storage at very low temperatures after baseline may trigger the brown tissue discoloration. In a similar study on storage trials of 15 Maris Piper stocks (including stock 20, 23 and 12 used in this present study) conducted by A. Briddon at Sutton Bridge Crop Science Research (SBCSR) in 2011, it was reported that BH symptoms were not detected directly after storage at very low temperatures (1.5 and 3.5^{o}C). However, he also reported that stock 23 (susceptible to BH) had ca. 6-fold higher BH incidence compared to stock 20 (susceptible to BH) and stock 12 (non-susceptible to BH) after packaging trials (Adrian Briddon, personal report, SBCSR). Also, those discolorations indicated during this present experiment were sometimes accompanied with brown scattered blotches similar to those symptoms observed in other physiological disorders named as brown centre, physiological necrosis, internal brown spot (Reeve, 1968). Craft *et al.* (1958) reported brown tissue discolorations in the

central tuber tissues developed due to the low temperature injury in potatoes cvs. Russet Rural and Kennebec packed in ventilated polyethylene bags and stored at 0^{o}C for ca. 4 months. Low temperature injury in potato tubers may be caused after storage at those cold temperatures slightly above the freezing point (0 - 2.5^{o}C), but the disorder may also be induced at 3.8 – 4.4^{o}C depended on the cultivar. Brown tissue discoloration initially occurs in vascular ring and the outer part of the tuber, but severe damage may result in grey and brown to black discoloration symptoms in the internal tuber tissue. However, its symptoms may overlap with other physiological disorders and pathogen diseases (Smith, 1978; Hooker, 1981; Wale *et al.*, 2008; UNECE 4th session note, 2011). Also, those tissue discolorations in the central tuber part observed from Craft *et al.* (1958) were similar to those of BH symptoms (dark internal discoloration in the central tuber part) and not symptoms of low temperature injury.

Mimicking the shelf-life conditions by transferring the tubers from a low initial storage temperature (1.5 or 3^{o}C) to 15^{o}C and storing them unwashed and unpacked in either air only or CA (10% CO_2) in sealed chambers with regular gas circulation, did not seem to show great differences in BH incidence as similar brown tissue discolorations were observed in both storage conditions used and yet these were more evident after storage in air (21% O_2). It has been reported that BH development can occur at any temperature by O_2 deprivation, but at low temperatures (5^{o}C) its development requires longer time. However, it is believed that the disorder may rapidly develop at 0 - 2.5^{o}C (Link *et al.*, 1932; O'Brien and Rich, 1976; Smith, 1978; Hooker, 1981; Wale *et al.*, 2008). In 1967, Lipton carried out an experiment by storing packed potatoes cv. White Rose (24 h after harvest) in CA chambers with 0.5, 1, 5 or 21% O_2 at various temperatures (5, 15, 20 and 22^{o}C) and reported that incidence of BH (10 - 13%) occurred after storage in very low O_2 levels (0.5 and 1% O_2) at 5 and 15^{o}C after 8-16 days of

storage. The author also reported no indications of BH in storage at 5 and 21% O_2 (air) at higher temperatures. Furthermore, Butchbaker *et al.* (1967), reported severe incidence of BH in unwashed packed potatoes cv. Kennebec after storage in CA chambers with 10% O_2 and 10-24% CO_2 at 4^oC for six months.. Those findings suggest that packaging combined with storage at lower O_2 levels and/or high CO_2 concentrations, poses also a significant barrier by further promoting the O_2 restriction (Beaudry, 2000; Fonseca *et al.*, 2000; Watkins, 2000). However, in this present experiment, it seems that O_2 was not really restricted in those chambers used where tubers were respiring normally somehow and respiration rate recorded by all stocks was mainly affected from the cold initial storage temperature, the efflux of CO_2 for those tubers held under CA conditions and a combination of both factors. Nevertheless, a relation between respiration rate and BH incidence could not be carried out as the former was measured placing three tubers in a jar using the Sable Respiratory System (see Chapter 3; session 3.4). After packaging trials on 15 Maris Piper stocks conducted in 2011, A. Briddon reported that BH incidence was 2-fold higher in punched polythene packed potatoes compared to those unpacked after storage at 20^oC for 13 days. He also concluded that initial storage temperature at 1.5^oC effectively increased BH incidence mainly in packed potatoes compared to 3.5^oC, but BH symptoms were accompanied with grey diffusion (suggested as total % of BH symptoms) were more evident in unpacked tubers (Adrian Briddon, personal report, 2011). It is obvious that low initial storage temperature may influence the incidence of BH-like symptoms. However, it is still unclear whether brown tissue discolorations indicated herein were stimulated or induced at very low initial storage temperature and then exacerbated in both storage conditions at 15^oC. The temperature and exposure period in which BH shows greater incidence cannot readily be predicted (Zhou *et al*, 2003) and therefore BH incidence was also evident on sampling days 0, but in general was randomly evident in all three sampling days (*viz.* 0, 3 and 7).

It is worth mentioning that when potatoes were transferred unwashed from the cold temperature in order to be sampled on day 0 at 15^{o}C, a prior 3 hour warming up for the tubers was required before sampling. It is well known that when potatoes transferred from a cooler to warmer temperature then condensation phenomenon occurs on their skin surface due to temperature difference and a restriction in O_2 diffusion arises due to the water film formed on their skin (Burton and Wigginton, 1970; Hooker, 1981; Pringle *et al.*, 1996, 2009; Wale *et al.*, 2008). However, during this experiment condensation did not occur.

Additional factors examined in this study were the possible effects of tuber size (namely length and maximum equatorial diameter) and tuber weight on the incidence of BH. Potato tubers from all three stocks examined were about same in size (ca. 102 mm in length, 70 mm in diameter) and weight (ca. 240 g). As previously mentioned, stock 23 (susceptible to BH) showed ca. 3-fold greater BH incidence compared to stock 20 (susceptible to BH), however, none of those dimensions measured nor the weight had any effect on BH incidence. On the other hand, a weak positive correlation was shown between BH incidence with tuber size and weight measured in affected and non-affected tubers of stock 20 after storage in air at 15^{o}C and initial storage at 1.5^{o}C for 8 weeks. Tuber length (mean= 104.9 mm), diameter (mean= 78.78 mm) and weight (mean= 313.2 g) were significantly greater in those affected tubers. Also, after 8 weeks of storage, tuber length was also greater (mean= 103.3 mm) in affected tubers of stock 20 stored at CA and have also been previously stored at 1.5^{o}C but no correlations between tuber length and BH incidence were observed. Once those brown tissue discolorations are initiated in the central part of the tuber (pith), it could be hypothesized that the narrowness of the pith which is positively associated with the tuber length as Tai and Misener (1994) pointed out and combined with the osmoregulation might have a possible role in tissue discoloration. However, longer tubers have narrow pith and pith cells localized in

various tuber parts (namely central, stem and bud end) may vary in size (Reeve *et al.*, 1971; Mogen and Nelson, 1986). From those findings it cannot truly be said that heavier and bigger sized tubers have greater incidence to brown tissue discoloration (Stewart and Mix, 1917). It has been reported that brown tissue discolorations may be developed in newly formed tubers as well (Van Denburgh *et al.*, 1980, 1986; Bussan, 2007; Zotarelli *et al.*, 2012) and therefore BH symptoms are more evident in medium sized tubers with ca. 60-70 mm in diameter (unpublished survey).

4.6.3. The effect of storage temperature and shelf-life conditions on the sugar content of potato stocks with different susceptibility to BH

Results indicated that flesh and heart tuber tissues derived from potato stocks with different susceptibility to BH may be well divided according to PCA on their sugar and phenolic content along storage time. Therefore, the effect of cold initial storage temperature (1.5^{o}C) on both sugar and phenolic contents was evident; this occurring from 8 to 20 weeks of storage. In general, chlorogenic acid and its isomers tended to be more accumulated in flesh samples of stock 12 (non-susceptible to BH) over storage time and also in those heart samples of stock 23 (susceptible to BH). Additionally, sugar content was highly accumulated in those samples of stock 23 when initial storage temperature occurred namely after 8 weeks of storage until the end of the storage period (20 weeks).

In terms of sugar content, unsurprisingly reducing sugars namely fructose and glucose were initiated and rapidly accumulated after baseline where tuber subsamples have initially been stored at 1.5^{o}C. This cold-induced phenomenon known as ‘low temperature sweetening’ (LTS) or ‘cold-induced sweetening’ (CIS) has been extensively studied and to date is still of

great interest (Muiller-Thurg, 1882; Pressey, 1969; Sowokinos, 2001; Malone *et al.*, 2006; Kaul *et al.*, 2010; Janksy *et al.*, 2014; Zhang *et al.*, 2014, etc.). Sucrose, the substrate for fructose and glucose biosynthesis, may either be catalysed by sucrose synthase or invertase enzymes. At cold storage temperatures, inactivation of invertase inhibitor leads to expression of invertase resulting in rapidly sucrose degradation to reducing sugar accumulation (Zhou and Solomos, 1998; Bologa *et al.*, 2003; Kumar, 2011). At baseline, flesh sucrose was significantly higher in stock 20 (susceptible to BH) compared to stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) and heart sucrose higher in those samples of stock 23. In general, fructose and glucose were ca. 2 times higher in heart than in flesh tissue of stock 20 and stock 12 with no really significant differences between those stocks and that pattern was followed after 8 weeks increasing from 16 to 20 weeks of storage. On the other hand, the cold temperature effect on sugar content was more evident in both control and discoloured flesh and heart tissue samples of stock 23 (susceptible to BH) from 8 to 20 weeks of storage. Although increased sugar content in potato tubers is related with brown to black discoloration; however, that is caused upon frying via Maillard reactions (Shallenberger *et al.*, 1958; Mackay *et al.*, 1990; Cottrell *et al.*, 1993; Stark *et al.*, 2003; McKenzie *et al.*, 2005; Zommick *et al.*, 2013). To date, no relation of reducing sugar accumulation and BH disorder has ever been reported. It has previously been pointed out that tuber tissues can be affected by other internal physiological disorders in potatoes such as brown centre, hollow heart and internal brown spot may accumulate reducing sugars (Davies, 1998; Bussan, 2007). In the present study, brown discolorations were mostly indicated in heart tissue samples of both susceptible to BH stocks (stock 20 and stock 23), but a few heart samples of stock 23 showed more intense brown to black tissue discoloration. It was observed that stock 23 which showed greater tissue discoloration (ca. 3-fold compared to stock 20) had also higher reducing sugar content compared with stock 20 and stock 12 (non-susceptible to BH) after 8 to 20 weeks of

storage. In fact, after 16 and 20 weeks of storage stock 23 had significantly higher fructose and glucose concentrations and yet the greatest BH incidence at that period of storage time. That high sugar accumulation in stock 23 was observed in both tissue samples suggesting that the whole tuber was affected after storage at low cold temperature. Sugar content may vary from cultivar to cultivar but it has been shown that it might be more accumulated in the pith indicating that its mobilization is more active towards this tuber area (Baijal and Van Vliet, 1966; Weaver *et al.*, 1978). When Weaver *et al.* (1978) examined the reducing sugar content in different tuber parts of six potato cultivars they reported sugar content variation between those cultivars after storage at 7^{o}C for 2 – 4 months and/or after reconditioning at 20^{o}C for 3 weeks; however, it was shown that cvs. Kennebec and White Rose had higher reducing sugar content in the core tuber part while Russet Burbank the lower. Dwelle and Stallknecht (1978) also reported slightly higher total and reducing sugar content in central pith tissue samples of Kennebec compared to Russet Burbank after storage at 1.7^{o}C. Those three potato cultivars seem to have different susceptibility to physiological disorders. O'Brien and Rich (1976) suggested that Russet Burbank cultivar is resistant to BH while according to Robinson and Secor (NDSU, 2014) this cultivar is susceptible to internal heat necrosis (IHN) and brown centre (BC) as Van Denburgh *et al.* (1980, 1986) has previously pointed out. Kennebec cultivar was found being susceptible to low temperature injury as Craft *et al.* (1958) observed brown tissue discoloration in the central pith part of packed tubers after storage at 0^{o}C for ca. 4 months and Butchbaker *et al.* (1967), severe BH symptoms after storage in CA chambers with 10% O_2 and 10-24% CO_2 at 4^{o}C for six months. Also, Lipton (1967) reported BH incidence in White Rose potato tubers after storage in 0.5 – 1% O_2 at 15 – 20^{o}C with ca. 2-fold lower glucose concentration in the outer and inner parts of the tubers compared to those held in air (21% O_2). Furthermore, Zhou and Solomos (1998) showed increase in sugar content of Russet Burbank potatoes in air at 1^{o}C, but strong inhibition after storage in 1.5%

O_2 at 1^oC due to hypoxia. However, in this present study sugars were much higher compared to those results previously published, but it seems that different storage conditions and storage temperature affect the sugar accumulation.

Additionally, it should be reminded that in the present study, potato tuber stocks have been initially stored at 1.5^oC from 8 to 20 weeks and sugar content studied at 15^oC every four weeks, thus storage time may have an effect on its accumulation (Butchbaker *et al.*, 1967; Spychalla and Desborough, 1990; Cottrell *et al.*, 1993). Moreover, storage at cold temperatures may lead to cellular stress response by negatively affecting the membrane lipid composition and subsequently leading to changes in fatty acid degradation, ion leakage thus greater membrane permeability and cell dysfunction are caused (Sowokinos *et al.*, 1985, 2001; Wills, 1989; Berkel *et al.*, 1994; Davies, 1998; Wismer *et al.*, 1998; Blenkishop *et al.*, 2004; Kumar, 2011). Shekhar *et al.* (1979) stated that membrane permeability is greater for tubers stored at cold temperatures than those held at higher. Lojkowska (1988) has pointed out that lipid content at post-wounding phase and after aging varied between different potato cultivars. Thus, potato stocks with different susceptibility to BH and similar physiological disorders may also show susceptibility to lipid peroxidation (Davies, 1998) as it has been reported that some potato cultivars with higher unsaturated fatty acid content have lower sugar accumulation (Spychalla and Desborough, 1990). From those findings, it could be hypothesized that reducing sugar may be increased due to brown tissue discolorations and be related with BC and similar disorders. Although, it is likely that tuber tissues showing more intense brown tissue discoloration may contain higher sugar content as it was observed for both tuber tissue samples of stock 23 (susceptible to BH), that cannot really highlight a relation with BH due to very low indication of dark brown to black symptoms indicated in this study and believed to be BH symptoms.

4.6.4. The effect of storage temperature and shelf-life conditions on the phenolic content of potato stocks with different susceptibility to BH

It is well known that the shikimate pathway is responsible for the aromatic amino acid biosynthesis namely phenylalanine, tyrosine and tryptophan (Herrmann, 1955; Dewick, 2002; Vogt, 2010; Payyavula *et al.*, 2012). Phenylpropanoid pathway is initiated by deamination of phenylalanine via the enzyme phenylalanine ammonia lyase (PAL) (Joos and Halbrock, 1992; Gerasimova *et al.*, 2005) generating a large amount of secondary metabolites including phenolic compounds such as hydroxycinnamic acids and flavonoids considered as a great source of antioxidants (Brown *et al.*, 2005). In terms of hydroxycinnamic acids, it is believed that chlorogenic acid which is a combination of caffeic and quinic acid comprises about 80 – 90% of the total phenolic content in potato tubers (Dao and Friedman, 1992) and along with its isomers neo-chlorogenic acid (3-O-caffeoyl-quinic acid) and crypto-chlorogenic acid (4-O-caffeoylquinic acid) may count up to 96 – 98% (Im *et al.*, 2008; Hamouz *et al.*, 2010).

In this experiment, among the chlorogenic acids studied chlorogenic acid (5-O-caffeoylquinic acid) was the major phenolic acid quantified followed by its isomers crypto- and neo-chlorogenic acid that showed appreciable quantities. Similar concentrations of both isomers have previously been reported in white fleshed potato cultivars (Lachman and Hamouz, 2005; Navarre *et al.*, 2011; Payyavula *et al.*, 2013). In contrast Ferndandez *et al.* (1996) observed that chlorogenic, neo- and crypto-chlorogenic acids have a quantity ratio of 8:5:1. Significant differences between flesh and heart tissue samples and potato stocks with different susceptibility to BH were shown. It should be noted that phenolic content quantification was carried out in flesh and heart samples according to potato susceptibility to BH and both control and discoloured samples were merged and averaged in the case of both

susceptible stocks 20 and 23 due to low BH incidence indicated. Chlorogenic acid accumulation varied between different tuber tissues and it has been reported that is more accumulated in the outer tuber parts than the inner with the peel and cortex accumulating the highest content (Craft *et al.*, 1958; Zucker and Levy, 1958; Dao and Friedman, 1992; Friedman, 1997). In general, its content may range from 33 to 12746 μg g^{-1} DW (Evers and Deußer, 2009) with the highest content accounting for coloured fleshed potatoes, in particular purple fleshed cultivars (Lewis *et al.*, 1998; Brown *et al.*, 2005; Andre *et al.*, 2007; 2009; Navarre *et al.*, 2011). Chlorogenic acid biosynthesis is not fully understood as several pathways have previously been proposed (Niggeweg *et al.*, 2004). In this study it was observed that caffeoyl-D-glucose which might be a precursor for chlorogenic acid synthesis via hydroxyl cinnamoyl D-glucose:quinate hydroxycinnamoyl transferase (HCGQT) (Shakya and Navarre, 2006) varied in content but according to PCA biplots it was shown that caffeoyl-D-glucose was mainly accumulated when chlorogenic acid and its isomers were highly accumulated as well. That might happen because caffeoyl-D-glucose was quantified against authentic standards of caffeic acid. According to Aksamit-Stachurska *et al.* (2008) this chlorogenic acid biosynthetic pathway seems to function in transgenic lines; however, it is believed that in Solanaceous species chlorogenic acid is synthesized from caffeic acid via the enzyme hydroxycinnamoylcoenzyme A quinate hydroxycinnamoyl transferase (HQT) (Andre *et al.*, 2009; Navarre *et al.*, 2013) and in their recent study Payyavula *et al.* (2014) concluded that HQT enzyme is responsible for chlorogenic acid biosynthesis. Furthermore, it is well documented that due to low storage temperature a cold-induced PAL enzyme triggers the biosynthesis of phenolic compounds that accumulate in response to stress (Cheynier *et al.* 2009). Potatoes contain ca. 40 – 50 PAL enzymes divided in two sub-families PAL1 and PAL2 (Andre *et al.*, 2009). Cheynier *et al.* 2009 has pointed out that low temperature effect

should trigger both PAL activity and those enzymes involved in the phenylpropanoid pathway in order the phenolic compounds to be accumulated. Low storage temperature also triggers the accumulation of reactive oxygen species (ROS) that negatively affect the cellular membrane integrity. Plants have the ability to react against those ROS developing membrane repair mechanisms, but this ability depends on many factors. It is well known that phenolic compounds have scavenging activities along with vitamin C against reactive oxygen species (ROS) (Brown *et al.*, 2005; Takahama *et al.,* 2002). Also, it has been reported that vitamin C in potatoes decreases at low storage temperature (Kawakami *et al.*, 2000; Dale *et al.*, 2003) and that scavenging system may be also lowered, thus leading to greater cell membrane degradation and lipid peroxidation. Further, cell membrane stability may negatively be affected by calcium deficiency (Palta, 2010). Although vitamin C and scavenging system properties were not examined in this present study, it may be hypothesized that non-affected tuber tissues may be adapted differently under low storage temperature (Purvis and Shewfelt, 1993) and also have greater scavenging activity. Therefore, it is believed that sugars may also affect the phenylpropanoid metabolism (Koch, 1996; Navarre *et al.*, 2011; Payyavula *et al.*, 2013) and due to low storage temperature effect on sugar content, carbon sources may be provided for further biosynthesis of phenolic compounds such as chlorogenic acid in potatoes (Kulen *et al.*, 2013). However this hypothesis has not clearly proved (Kumar, 2011). Craft *el al.* (1958) reported that total phenolic content in packed potato tubers cvs. Kennebec and Russet did not show any increase after storage at 0, 4 or 13^{o}C for 5 months but that increase was due to brown tissue discoloration caused by low temperature injury. Later, Hasegawa *et al.* (1966) stated that increased chlorogenic acid content in the pith of potato cvs. Kennebec and Katahdin was due to sugar accumulation after storage at 4^{o}C. More recent, Zhou and Solomos (1998) showed a 3-fold increase in chlorogenic acid after storage at 1^{o}C but inhibition of both sugar and chlorogenic content due to hypoxia (1.52% O_2) at 1^{o}C was

observed. According to the PCA biplots, it seems that chlorogenic acid and its isomers were mostly accumulated in the heart samples of stock 23 and that was evident over storage period even though no indications of tissue discolorations were observed during baseline but there were no really differences between flesh and heart samples of stock 23. Further, both chlorogenic isomers were significantly higher in those flesh samples of stock 12 (non-susceptible to BH) (up to 8.6 μg g^{-1} DW) with low accumulation in its heart samples and a similar trend was followed by stock 20 (susceptible to BH). A chlorogenic isoform was identified and quantified in the middle, stem and apical tuber part of Korean potato cultivars ranging from 1.6 – 2, 2.5 – 3.4 and 13.8 – 27.6 μg g^{-1} FW respectively (Im *et al.*, 2008). On the other hand, it seems that flesh chlorogenic acid content was similar for all three stocks analysed after 8 weeks of storage when the initial storage temperature (1.5^{o}C) occurred and that was probably because no discoloration in flesh tissue were indicated. In contrast, chlorogenic acid was significantly higher in those heart samples of stock 23 which showed greater BH incidence. Im *et al.* (2008) reported that chlorogenic acid was lower in the inner tuber part (pith) than in the outer. Additionally sugar content was affected by the initial storage temperature and that was observed in all three stocks, but it was more evident in both tissue samples of stock 23.

The amino acid phenylalanine which is the precursor for phenolic compound biosynthesis it was more expressed in those heart samples of stock 23 and that was more evident after 16 and 20 weeks of storage where stock 23 showed greater BH incidence. Also, tryptophan content varied between the stocks analysed but was significantly lower in stock 12 (non-susceptible to BH), but was increased in heart samples of stock 20 and stock 23 after 16 and 20 weeks of storage and yet according to the biplots, tryptophan was mainly accumulated in those discoloured heart samples of stock 23 after 20 weeks of storage. Yao *et al.* (2005)

reported that both phenylalanine and tyrosine amino acids may positively be activated by tryptophan. In terms of tyrosine, its content varied across storage time but after 8 and 16 weeks of storage it significantly increased in heart samples of stock 20 (susceptible to BH) and a similar trend was followed by those heart samples of stock 23 (susceptible to BH) after 16 and 20 weeks where BH incidence was greater as well. Generally, Maris Piper potatoes contain ca. 450 – 1190, 130 - 940 and 340 - 1190 μg g^{-1} DW phenylalanine, tryptophan and tyrosine after storage at room temperature ranging from 10 – 20^{o}C (Davies, 1976). However in this study tyrosine and phenylalanine were much lower in concentration.

In terms of tissue discoloration, it was previously mentioned that stock 23 showed ca. 3-times higher BH incidence compared to stock 20 (susceptible) and stock 12 (non-susceptible) and that was more apparent after 16 and 20 weeks of storage. In general, less and more intense brown tissue discolorations were indicated in the heart tissue samples. According to the results, after 16 and 20 weeks most of the phenolic compounds and reducing sugars quantified were mostly accumulated in those heart samples of stock 23. Further, PCA biplots performed on heart tissue samples showed that after 16 and 20 weeks of storage chlorogenic acid and its isomers were mainly accumulated in control heart samples of stock 23 whereas reducing sugars grouped in those discoloured heart samples. Also the general PCA on heart samples revealed that indeed control heart samples of stock 23 were well grouped according to chlorogenic acid and its isomers while sugars, phenylalanine and tyrosine were those variables differentiating the discoloured heart samples. The biochemistry of the BH disorder is scarce. Initially, Bartholomew (1916) reported that tyrosinase is activated at high temperatures (< 38^{o}C) thus tyrosine is being oxidized resulting in black tissue discoloration. Later, Reeve (1968) after histochemical studies on affected tuber tissues showing BH symptoms concluded that both chlorogenic acid and tyrosine were present. It is well known

that both chlorogenic acid and tyrosine are the major phenolic compounds present in potato tubers with tyrosine being more accumulated in the inner part (Craft *et al.*, 1958; Reeve, 1968) and both compounds are adequate substrates for enzymatic oxidation; tyrosine by polyphenol oxidase (PPO or tyrosinase) and chlorogenic acid by PPO or peroxidase (POD) (Takahama *et al.*, 2004; Adams, 2007). Also, black discoloration is mainly caused by tyrosine oxidation while chlorogenic acid oxidation results in brown pigments (Adams *et al.*, 2007). However, there is confusion between substrates and enzyme activity related to tissue discoloration and the final colour of the oxidation products (Werij *et al.*, 2007). Chlorogenic content was much higher than that of tyrosine, thus ideally it could be hypothesized that the former compound has greater possibility to be involved in tissue discoloration. However, it was more accumulated in those control heart samples of stock 23. It is still unclear if tyrosine or chlorogenic acid or other phenolic compounds that were not quantified contributed to those brown tissue discolorations indicated in this study and it is more likely that caused due to initial storage temperature as BH incidence was observed in both storage conditions and yet was greater in those tubers stored in air than in CA (10% CO_2).

4.7. Conclusions

In conclusion, the cold initial temperature was the main factor influencing both respiration rate and compositional changes in potato tubers from stocks with different susceptibility to BH. Respiration rate could not be related with BH incidence but a similar trend was followed by all stocks analysed where CO_2 production was greater in those tubers initially stored at 1.5 than 3^{o}C and this trend was more evident when tubers held 10% CO_2. BH incidence was not observed at baseline but brown tissue discolorations in the heart part of both susceptible stocks (20 and 23) were indicated after 8 weeks of storage at 1.5 or 3^{o}C.

Similar brown tissue discolorations were observed in both air and CA storage conditions suggesting that 10% CO_2 did not affect the BH incidence as it was expected and yet tissue discoloration was mainly observed in those tubers stored in air and initially stored at 1.5^{o}C suggesting that O_2 was not really depleted. It seems that initial storage temperature had a greater effect on stock 23 which also showed significantly higher BH incidence compared to stock 20 (susceptible to BH) and stock 12 (non-susceptible to BH). Stock 23 showed greater sugar accumulation in both flesh and heart tissue samples suggesting that the whole tuber was affected and yet higher sugar content in discoloured samples was observed and that could be a biomarker of susceptibility. It was also observed that amino acids and chlorogenic acid tended to be more accumulated in those heart samples of stock 23 which had greater BH incidence and increased when BH incidence was higher as well indicating differences in gene expression between the potato stocks with different susceptibility to BH. Also, it seems that brown tissue discolorations were triggered and induced due to low storage temperature, but the fact that BH incidence was very low during this experiment it cannot be concluded that those tissue discolorations observed were precursors of BH as the temperature and time exposure in which BH is increased could not be predicted.

CHAPTER FIVE:

The effect of storage temperature and shelf-life conditions on the physiological and biochemical changes of potato stocks with different susceptibility to blackheart disorder (Year 2: 2012-2013)

5.1. Introduction

Similar experiments to that of year 1 (2011-2012) were conducted in this year 2 study with some modifications. Three potato stocks cv. Maris Piper with different susceptibility to BH (two susceptible stocks and one non-susceptible) were also selected and used for physiological and biochemical analysis. In this study, potato tubers were initially stored at 1.5^{o}C only. Two experiments were conducted within a period of seven months. The first experiment (December 2012 – May 2013) was similar to that of year 1, but in year 2 potato stocks were transferred from a cold temperature (1.5^{o}C) to 20^{o}C for shelf-life evaluation where storage took place in air only (21% O_2) and tubers sampled at two sampling days (0 and 7). The second experiment was carried out in June 2013 by storing potato tubers under various gas combinations at 20^{o}C for 14 days and only physiological assessments were evaluated.

5.2. Materials and methods

Real time respiration rate measurement and sample preparation were provided in Chapter 3: Section 3.4 and 3.5 respectively. In Chapter 3; Section 3.6, sample extraction and quantification for non-structural carbohydrate content (sugars) and phenolic content analysis was described.

5.3. A) Experiment 1

5.3.1. Respiration rate

Tuber respiration rate for potatoes cv. Maris Piper stock 7 (susceptible to BH), stock 12 (susceptible to BH) and stock 3 (non-susceptible to BH) was analysed using ANOVA. Factors included the potato stock [stock 7, stock 12 (both susceptible to BH) and stock 3 (non-susceptible to BH)], outturns [baseline, week 4, week 8, week 12, week 16 and week 20 (OT)] on sampling days (day 0 and 7) and corrected replication (n = 3). The structure used was STOCK*OT*DAY.

In general, stock 3 (non-susceptible to BH) scored the highest respiration rate with mean of 7.62 ml CO_2 kg^{-1} h^{-1} compared to stock 7 and stock 12 (both susceptible to BH) (means = 5.32 and 5.14 ml CO_2 kg^{-1} h^{-1} respectively). CO_2 rates were ca. 2 times significantly higher in stock 3 after 4, 8 and 12 weeks of storage (Fig. 5.1), However, there were no significant differences between stock 7 and stock 12 over storage time and their respiration rate ranged from 4.22 to 6.41 and 4.72 to 6.79 ml CO_2 kg^{-1} h^{-1} respectively. According to Table 5.1 respiration rate was ca. 2 times higher for those tubers of stock 3 (non-susceptible to BH) when compared to both susceptible stocks 7 and 12 on sampling day 0. However, there were no significant differences between the susceptible stocks 7 and 12 neither on day 0 nor on day 7. Therefore, on sampling day 7, the respiration rate was almost the same for all stocks with no significant differences observed (Table 5.1).

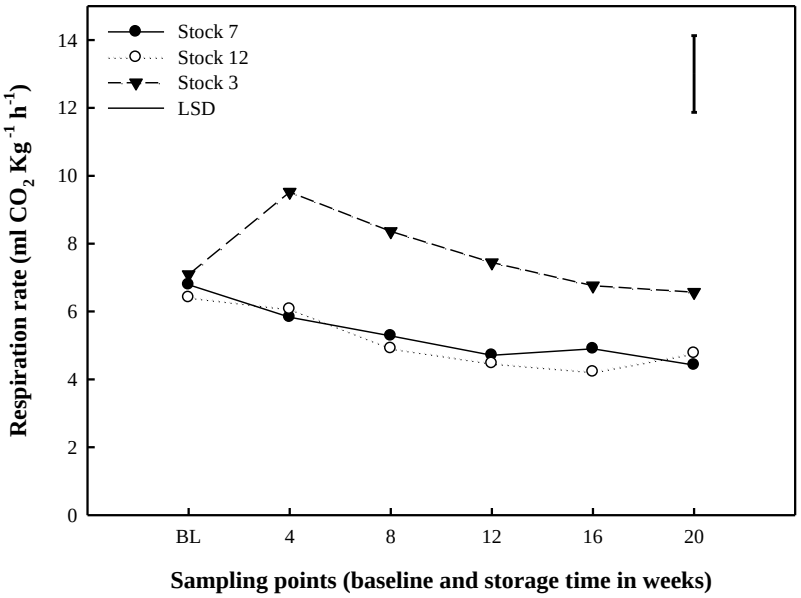


Figure 5.1 Respiration rate (ml CO_2 kg^{-1} h^{-1}) of potato stocks cv. Maris Piper [stock 7 (susceptible to BH), stock 12 (susceptible to BH) and stock 3 (non-susceptible to BH)] recorded after baseline (BL) and storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks. Values are means (n = 3). General LSD is shown.

Table 5.1 Mean respiration rate (ml CO_2 kg^{-1} h^{-1}) of potato tubers cv. Maris Piper stock 7 (susceptible to BH), stock 12 (susceptible to BH) and stock 3 (non-susceptible to BH) recorded after baseline and storage in air at 20^{o}C on sampling days 0 and 7 (Appendix C; Table 1).

STOCK	**SAMPLING DAY**	
	0	7
Stock 7	5.77	4.87
Stock 12	5.69	4.59
Stock 3	10.12	5.13

5.3.2. The incidence of blackheart

Results of BH incidence for potato tubers cv. Maris Piper stock 7 (susceptible to BH) stock 12 (susceptible to BH) and stock 3 (non-susceptible to BH) across storage time are

tabulated in Table 5.2. Tubers were sampled at two different sampling days (0 and 7) each four weeks at 20^{o}C (n = 324 in total). Tubers were initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks. Incidence of BH was initially observed after the baseline (9.3%) peaking in March (13%) and in May (13%) after 12 and 20 weeks of storage, respectively (Table 5.2). Example figures of tubers with tissue discoloration indications are present in Appendix A; Figure 2.1 – 2.5.

According to Table 5.3, it was shown that stock 7 (susceptible to BH) had a greater susceptibility to BH compared to stock 12 (susceptible to BH) and stock 3 (non-susceptible to BH) (Table 5.3). However, Chi Square test results showed no significant differences in BH incidence between stock 7 and stock 12 over storage time (Table 5.4). The only significant differences in BH incidence were found between the susceptible stocks (7 and 12) when compared to the non-susceptible to BH stock 3 (Table 5.4).

Table 5.2 Total percentage of BH incidence for potato tubers cv. Maris Piper stock 7, stock 12 (susceptible to BH stocks) and stock 3 (non-susceptible to BH stock) after 154 days of storage in air.

Sampling point	Month	Year	Tubers used	BH incidence (%)
Baseline (days 0 and 7)	December	2012	54	9.3
Week 4 (days 28 and 35)	January	2012	54	9.3
Week 8 (days 56 and 63)	February	2013	54	1.9
Week 12 (days 84 and 91)	March	2013	54	13
Week 16	April	2013	54	11.1

(days 119 and 126) Week 20 (days 147 and 154)	May	2013	54	13
TOTAL			324	9.6

Table 5.3 Percentage BH incidence of potato cv. Maris Piper stock 7, stock 12 (susceptible to BH stocks) and stock 3 (non-susceptible to BH) after baseline and storage in air at 20°C. Tubers were initially stored at 1.5°C for 4, 8, 12, 16 and 20 weeks.

	Stock 7		Stock 12		Stock 3	
	Day 0	Day 7	Day 0	Day 7	Day 0	Day 7
Sampling point	BH %					
Baseline	0	22.2	0	22.2	0	0
Week 4	22.2	11.1	11.1	11.1	0	0
Week 8	0	11.1	0	0	0	0
Week 12	11.1	11.1	44.4	0	0	0
Week 16	11.1	33.3	11.1	11.1	0	0
Week 20	22.2	22.2	11.1	11.1	0	0
TOTAL %	11.1	18.5	13.0	9.3	0	0

Table 5.4 Chi-square test results of BH incidence for potato tubers cv. Maris Piper between stock 7, stock 12 (susceptible to BH stocks) and stock 3 (non-susceptible to BH) after storage in air at 20°C over storage time (baseline included). Tubers were initially stored at 1.5°C for 4, 8, 12, 16 and 20 weeks ($P < 0.05$) (Appendix C; 2.1 – 2.3).

Storage time	BH %		
All outturns	Stock 7	Stock 12	*Probability*
	14.8	11.1	0.418
All outturns	Stock 7	Stock 3	*Probability*
	14.8	0	< 0.001
All outturns	Stock 12	Stock 3	*Probability*
	11.1	0	0.0004

5.3.2.1. Effect of tuber respiration rate, tuber size and tuber weight on BH incidence

'Two-sample' t-tests were used in order to estimate a possible link between tuber respiration rate and BH incidence. However, no significant differences were observed comparing tuber respiration rate with BH incidence for stock 7 and 12, after baseline and 4, 8, 12, 16 and 20 weeks of storage (Table 5.5). According to Table 5.5, there were no significant effects of tuber size and weight on the incidence of BH for those tubers of stock 7 (Table 5.5). A significant effect of tuber weight on BH incidence for those tubers of stock 12 was observed after 16 weeks of storage (P = 0.007) (Table 5.6). Affected tubers of stock 12 had greater weight (mean = 356.2 g) compared to unaffected ones (mean = 281.6 g). Therefore, a weakly positive correlation has been found between tuber weight and BH incidence (r = 0.58) (Table 5.7).

Table 5.5 Two-sample T-test results for BH incidence with respiration rate, tuber length, diameter and weight of potato cv. Maris Piper stock 7 (susceptible to BH) after baseline and storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks (P < 0.05) (Appendix C; Table 3.1 – 3.20).

Sampling point	CO_2	Length (mm)	Diameter (mm)	Weight (g)
Baseline	0.797	0.893	0.188	0.759
Week 4	0.704	0.494	0.969	0.842
Week 8	-	-	-	-
Week 12	0.617	0.742	0.882	0.675
Week 16	0.930	0.464	0.106	0.216
Week 20	0.941	0.464	0.228	0.340

Table 5.6 Two-sample T-test results for BH incidence and respiration rate, tuber length, diameter and weight of potato cv. Maris Piper stock 12 (susceptible to BH) after baseline and storage in air at 20°C. Tubers were initially stored at 1.5°C for 4, 8, 12, 16 and 20 weeks ($P < 0.05$) (Appendix C; Table 4.1 – 4.20).

Sampling point	CO_2	Length (mm)	Diameter (mm)	Weight (g)
Baseline	0.904	0.863	0.906	0.507
Week 4	0.918	0.516	0.144	0.341
Week 8	-	-	-	-
Week 12	0.091	0.758	0.23	0.551
Week 16	0.529	0.058	0.409	0.007*
Week 20	0.468	0.312	0.962	0.626

Table 5.7 Correlation matrix comparing tuber length, diameter and weight with the BH incidence for potato cv. Maris Piper stock 12 after storage in air at 20°C. Tubers were initially stored at 1.5°C for 16 weeks *($P < 0.05$) (Appendix C; Table 4.21).

	BH	Length (mm)	Diameter (mm)	Weight (g)
BH	-	0.3838	0.0581	0.5723*
Length		-	-0.1786	0.7699
Diameter			-	0.2314
Weight				-

5.3.3. Biochemical assessments

5.3.3.1. Non-structural carbohydrates analysis

Fructose, glucose and sucrose, the major sugars present in potato tubers, were identified and quantified in flesh and heart tissue of discoloured and control samples of stock 7 (susceptible to BH). Also, matched control samples of stock 3 (non-susceptible to BH) were also included.

Fructose

In general, fructose content was ca. 2-fold higher in heart than in flesh tissue samples of all stocks sampled (61.6 and 35.6 mg g^{-1} DW respectively). At baseline, the concentration of fructose was significantly higher in BC (brown center) heart compared to BC flesh and BCL (brown center light) samples. However all those affected samples had significantly lower fructose compared to control samples of stock 7 and stock 3 as well. Heart fructose of stock 3 was ca.2-times greater (50.6 mg g^{-1} DW) compared to its flesh samples and yet ca. 2-3 times higher compared to those samples of stock 7 (Fig. 5.2). There were no significant differences in fructose content between both stocks analysed from 4 weeks to the end of storage time (Fig. 5.2).

Glucose

Similarly, glucose content was generally ca. 2 higher in heart than in flesh tissue samples (61.9 and 35.2 mg g^{-1} DW). At baseline BCL heart samples contained ca. 2-fold greater glucose (54 mg g^{-1} DW) compared to BC heart samples (24.2 mg g^{-1} DW), however, there were no significant differences between BCL and control heart samples of stock 7 and control samples of stock 3 (non-susceptible to BH) (Fig. 5.3). Flesh content in controls flesh samples of stock 7 was significantly higher than affected samples and flesh samples of stock 3 at baseline. From 4 to 20 weeks of storage, a similar pattern was followed by all those samples of stock 7 where heart glucose was ca. 2-fold significantly higher than in flesh tissue. This trend (heart > flesh glucose) was also observed in those samples of stock 3 after 4 and 16 weeks of storage. After 8 weeks of storage there was a significant decrease in flesh and heart glucose content of stock 3 (11.7 and 14 mg g^{-1} DW respectively) (Fig. 5.3).

Sucrose

In terms of sucrose, there were no significant differences between flesh and heart tissue samples regardless the stock. However, stock 3 (non-susceptible to BH) had significantly lower sucrose content along storage time compared to stock 7 (Fig. 5.4). At baseline and after 4 weeks of storage, affected samples of stock 7 contained significantly higher sucrose than those control samples of stock 7 and stock 3. Sucrose content was similar for those affected and control samples of stock 7 after 12, 16 and 20 weeks of storage (Fig. 5.4).

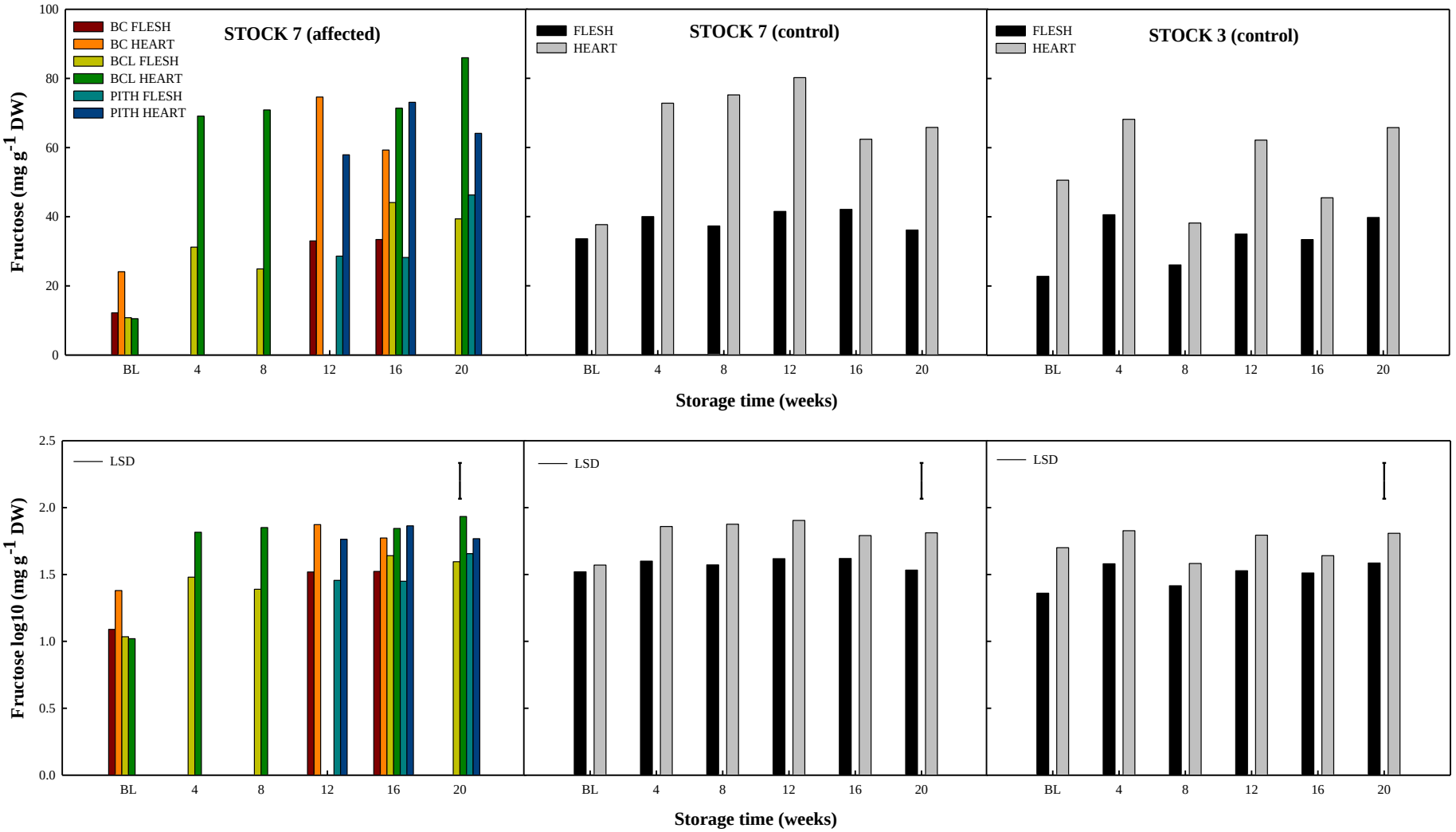


Figure 5.2 Fructose unlogged and logged10 concentrations (mg g^{-1} DW) in flesh and heart tissue of potato tubers Maris Piper stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) at baseline and after storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks. Values are means (n = 3). General LSD is shown (Appendix C; Table 5.1 – 5.2)

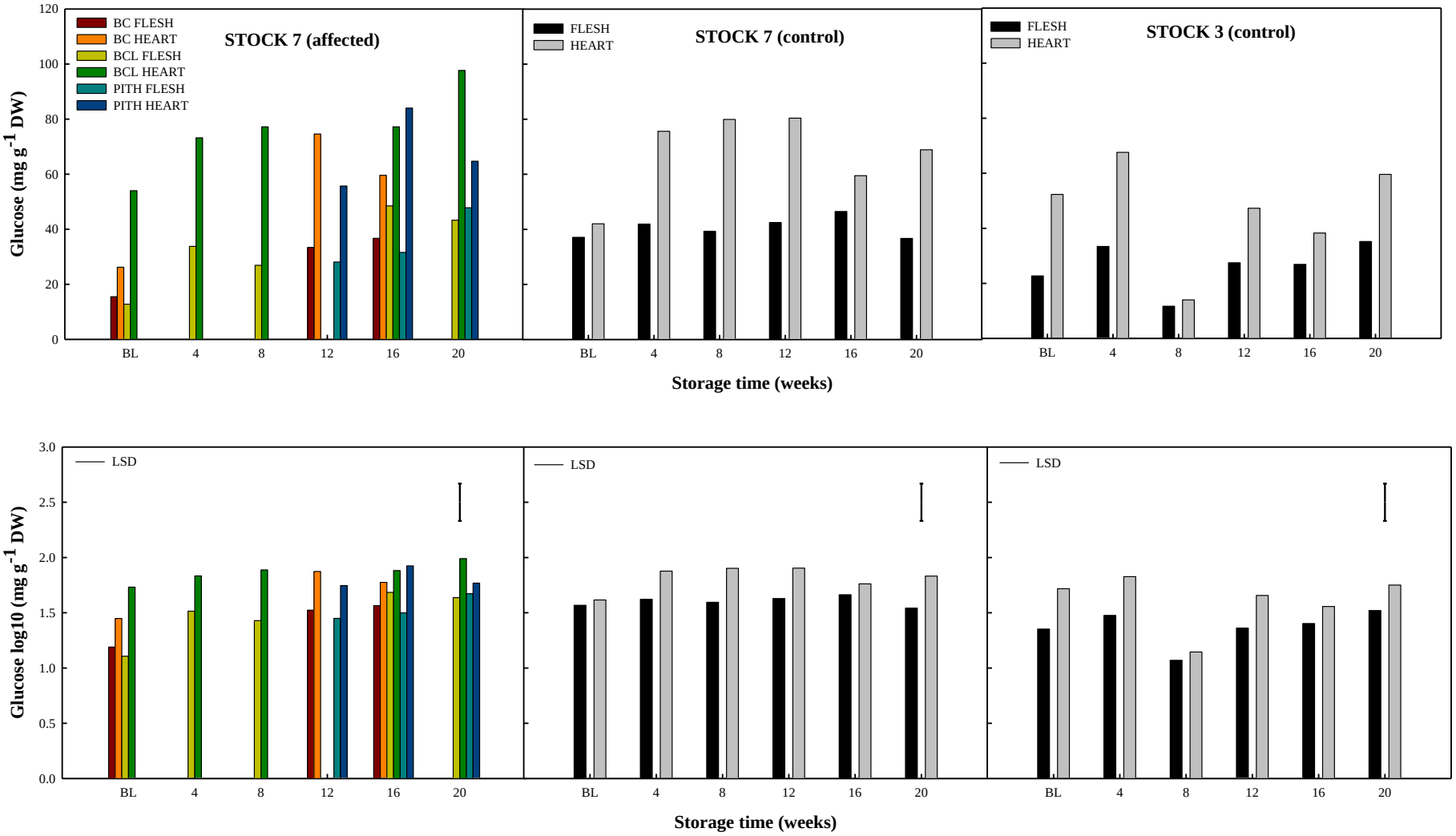


Figure 5.3 Glucose unlogged and logged10 concentrations (mg g^{-1} DW) in flesh and heart tissue of potato tubers Maris Piper stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) after baseline and storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks. Values are means (n = 3). General LSD is shown (Appendix C; Table 5.3 – 5.4).

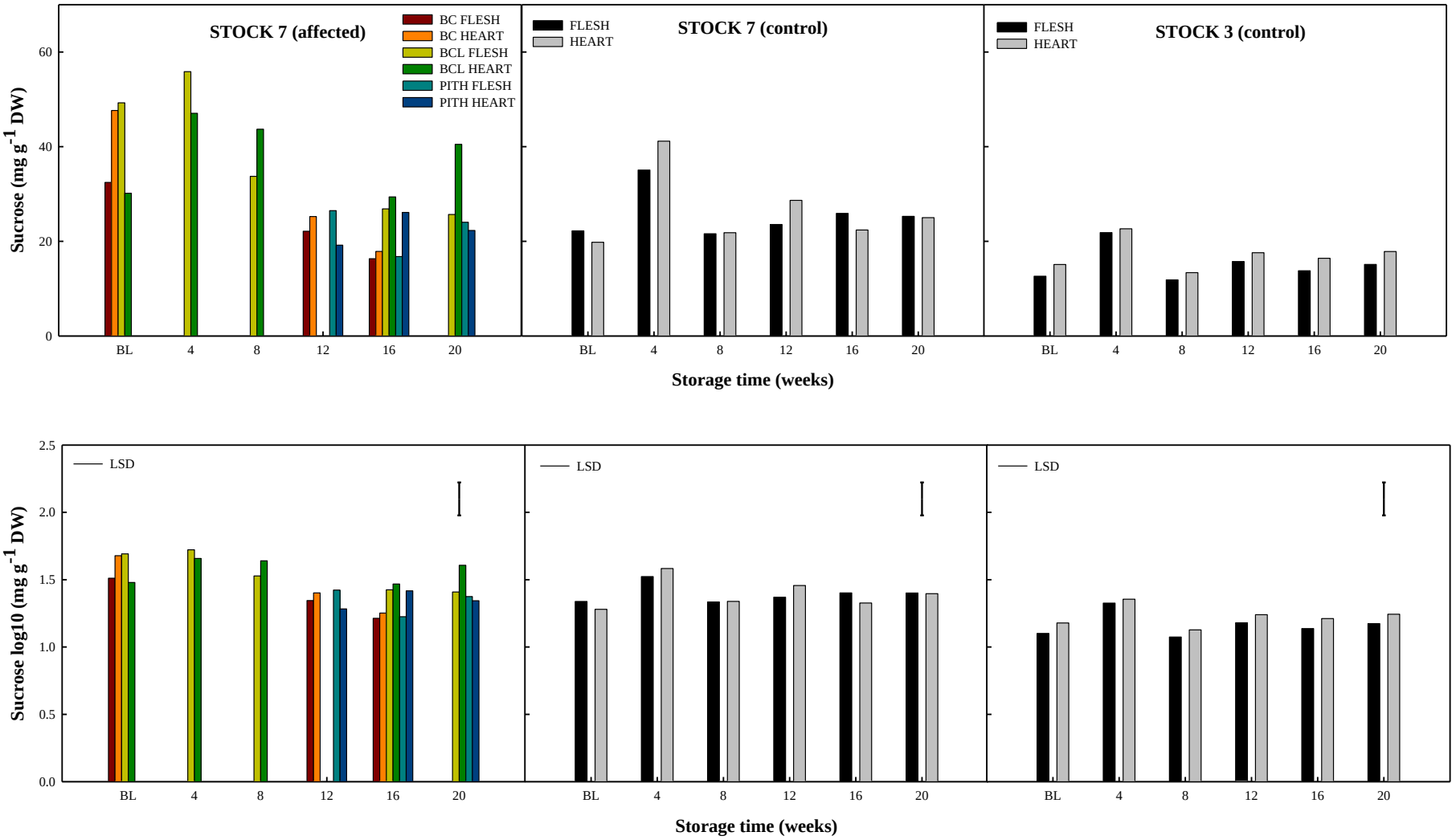


Figure 5.4 Sucrose unlogged and logged10 concentrations (mg g^{-1} DW) in flesh and heart tissue of potato tubers Maris Piper stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) after baseline and storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks. Values are means (n = 3). General LSD is shown (Appendix C; Table 5.5 – 5.6).

5.3.3.2 Phenolic content analysis

Hydroxycinnamic acid derivatives

Results indicated that chlorogenic acid showed greater abundance of all hydroxycinnamic acids examined in potato tissue samples. At baseline and after 4 weeks of storage, a similar trend was followed by affected and control samples of stock 7 where heart chlorogenic acid was higher compared to flesh samples. Heart samples with BCL discoloration had significantly higher chlorogenic acid concentration at baseline (1204 μg g^{-1} DW) and also after 4 weeks of storage along with control heart samples of stock 7 (919 and 954 μg g^{-1} DW respectively) (Fig. 5.5). After 12 weeks of storage heart samples with pith discoloration had greater chlorogenic acid content (911 μg g^{-1} DW). However, after 16 weeks, BC and pith samples had significantly lower chlorogenic acid (Fig. 5.5). In terms of stock 3, oppositely chlorogenic acid was ca. 2 times significantly higher in flesh than in heart samples (422-583 and 152-217 μg g^{-1} DW respectively) at baseline and after 4 and 12 weeks of storage. However, after 8, 16 and 20 weeks chlorogenic acid content was lower and there were no differences between flesh and heart samples of stock 3 (Fig. 5.5).

Chlorogenic acid isomers neo- and crypto-chlorogenic acid were also identified and quantified. Neo-chlorogenic acid was ca. 4 times higher in flesh tissue of stock 3 when compared to stock 7 (Fig. 5.6). Flesh neo-chlorogenic acid content of stock 3 was significantly increased at baseline (7.87 μg g^{-1} DW), decreased after 4 weeks of storage (3.47 μg g^{-1} DW) and then started increasing again along the storage time. According to Figure 5.6, flesh tissue samples had always higher neo-chlorogenic content compared to heart tissue over storage time and that was obvious in both stocks (Fig. 5.6). A similar trend followed where crypto-chlorogenic acid concentration was ca. 2 times higher in flesh tissue of stock 3 (non-susceptible) compared to stock 7 (susceptible to BH) (Fig. 5.7). In contrast, crypto-

chlorogenic acid was 2 times lower in heart tissue of stock 3 compared to stock 7. After 12 weeks of storage flesh crypto-chlorogenic acid was significantly higher in stock 3 (66 µg g^{-1} DW) and also in pith heart samples of stock 7 (54.7 µg g^{-1} DW). After 16 weeks of storage, there was a significant decrease of crypto-chlorogenic acid in control flesh samples of stock 7 (5.8 µg g^{-1} DW). Furthermore, after 20 weeks of storage heart crypto-chlorogenic acid of pith samples of stock 7 was significantly higher (49 µg g^{-1} DW) (Fig, 5.7).

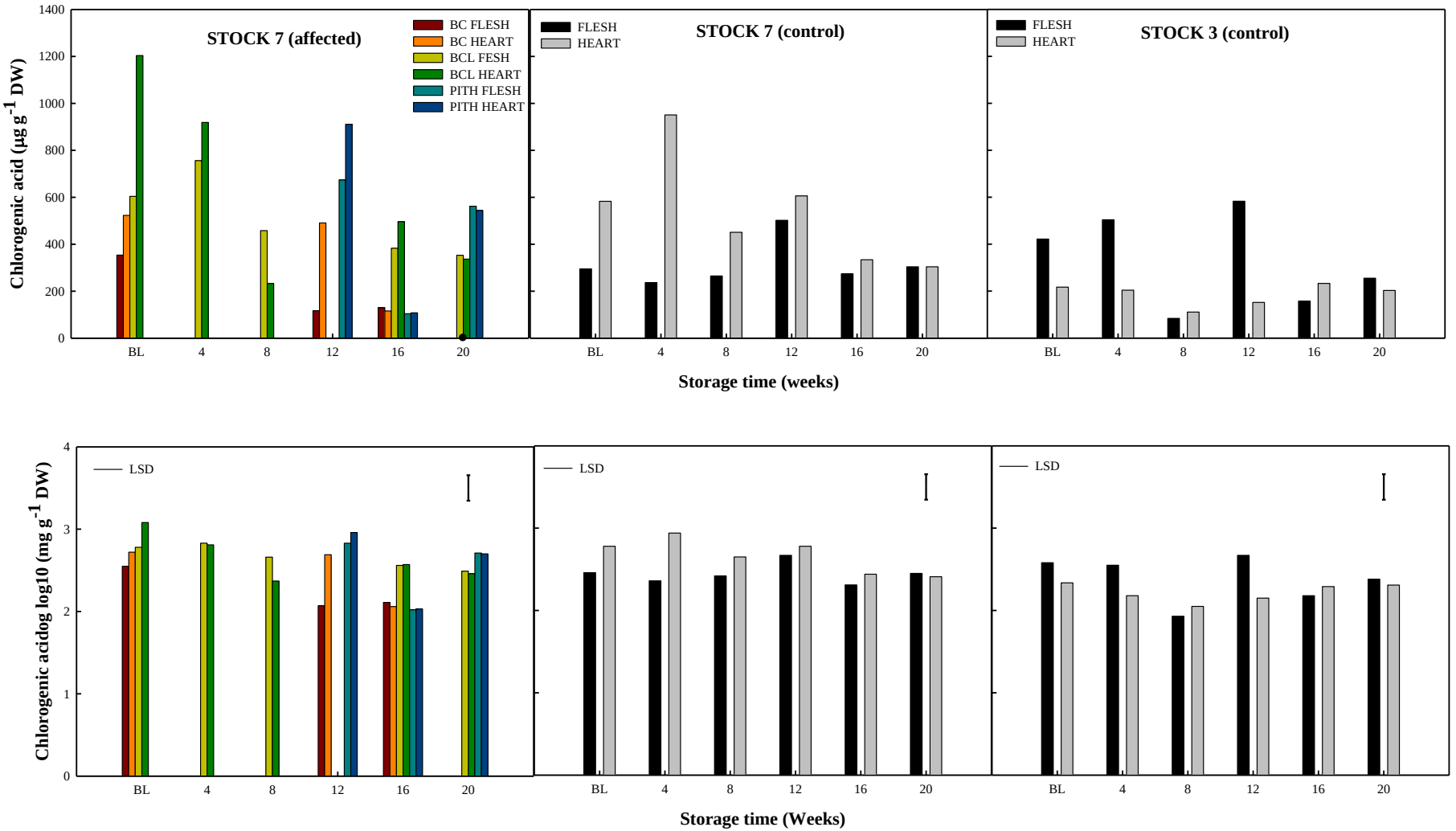


Figure 5.5 Chlorogenic acid unlogged and logged10 concentrations (µg g^{-1} DW) in flesh and heart tissue of potato tubers cv. Maris Piper stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) after baseline and storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks. General LSD is shown (Appendix C; Table 6.1 – 6.2).

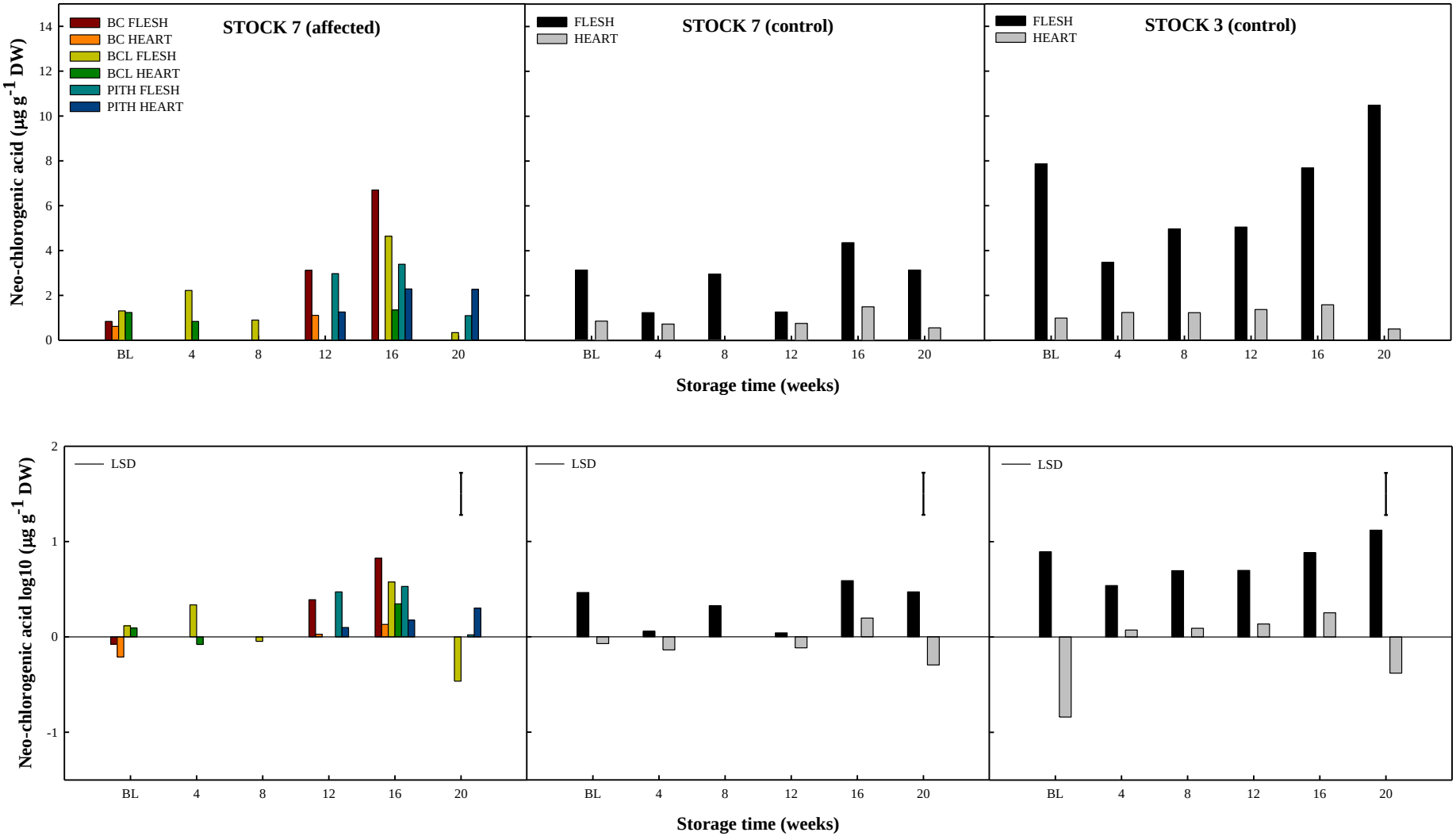


Figure 5.6 Neo-chlorogenic acid unlogged and logged10 concentrations (µg g^{-1} DW) in flesh and heart tissue of potato tubers cv. Maris Piper stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) after baseline and storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks. General LSD is shown (Appendix C; Table 6.3 – 6.4).

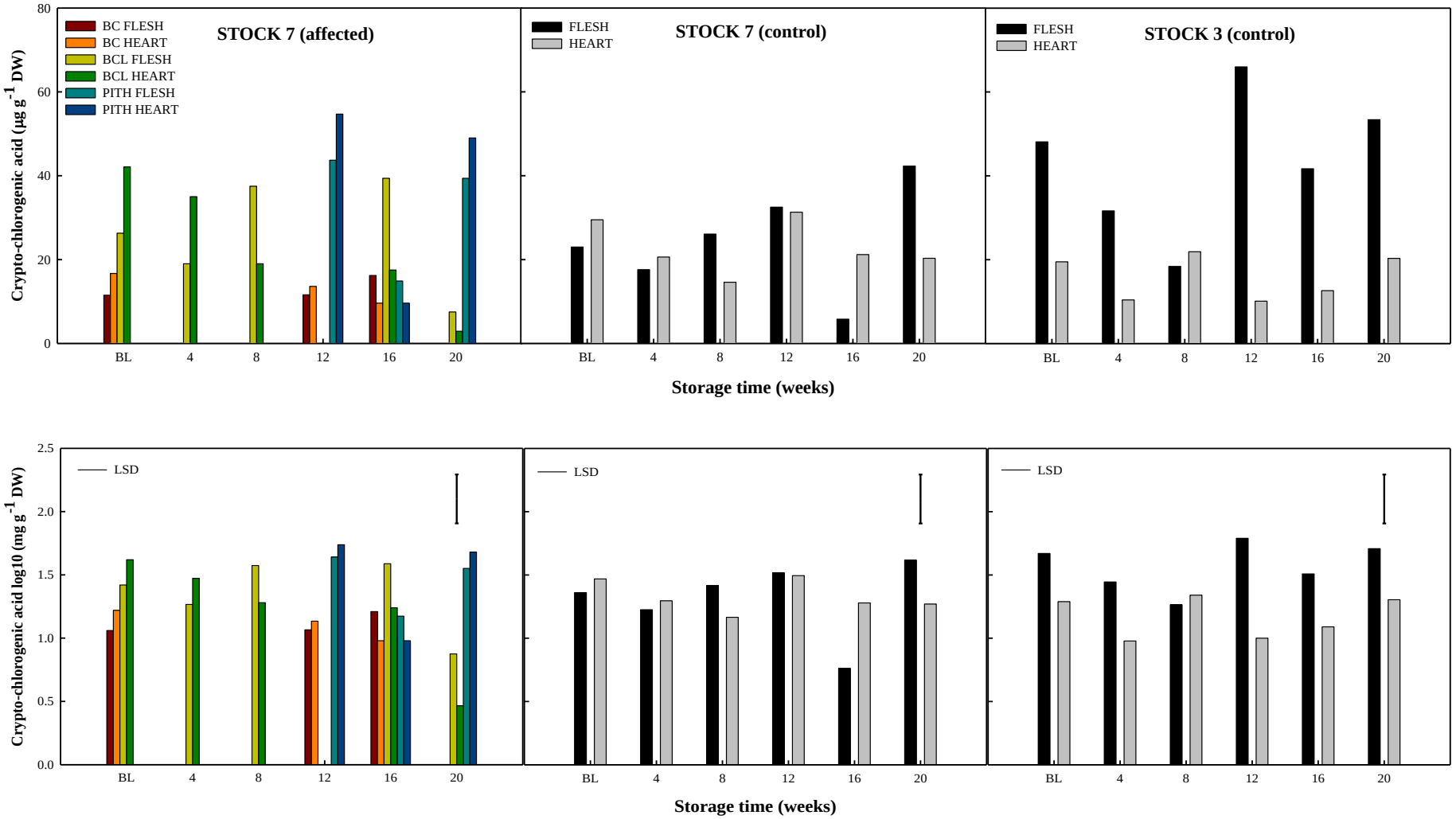


Figure 5.7 Crypto-chlorogenic acid unlogged and logged10 concentrations (µg g^{-1} DW) in flesh and heart tissue of potato tubers cv. Maris Piper stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) after baseline and storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks. General LSD is shown (Appendix C; Table 6.5 – 6.6).

Flavonols

According to Figures 5.8 and 5.9, both flavonols; quercetin-3,4-O-diglucoside and rutin respectively, followed a similar pattern where they were ca. 2-3 times more accumulated in stock 3 (non-susceptible to BH) when compared to stock 7 (susceptible to BH) irrespective of the tissue sampled. However, both flavonols varied in content among storage time. The highest quercetin-3,4-O-diglucoside concentrations were observed in those samples of stock 3 after 4 and 8 weeks of storage (12.8 and 12.2 µg g^{-1} DW respectively) (Fig. 5.8). Similarly, after 4 and 8 weeks of storage stock 3 contained the highest rutin concentrations ranging from 96.4 and 111.5 µg g^{-1} DW respectively (Fig. 5.9).

Aromatic amino acids

In terms of the three amino acids quantified, no statistical analysis was performed for tyrosine due to high number of missing values. However, significant differences in phenylalanine and tryptophan content were shown. In general, phenylalanine was greater in heart tissue samples but significantly higher only in those samples of stock 7. At baseline and after 16 weeks of storage, BCL heart samples had the highest phenylalanine content (29.68 µg g^{-1} DW respectively). Furthermore, high phenylalanine content was also observed in heart samples with BC and pith discoloration and also in control heart samples of stock 7 after 16 weeks of storage (28.72, 21.44 and 25.24 µg g^{-1} DW respectively). Also, the lowest phenylalanine concentrations were shown in flesh and heart samples with BC after 12 weeks of storage (7.99 and 7.06 µg g^{-1} DW respectively). In terms of stock 3 (non-susceptible to BH) there were no significant differences in phenylalanine content between flesh and heart samples over storage time ranging from 8.44 - 11.31 and 11.58 - 14.79 µg g^{-1} DW respectively (Fig, 5.10). Tryptophan was only significantly higher in heart samples with BCL

after 8 and 16 weeks of storage (243 and 221 μg g^{-1} DW respectively). No significant differences between control samples of stock 7 and stock 3 were observed (Fig. 5.11).

No statistical analysis was carried out for caffeic, *p*-coumaric and ferulic acids because those compounds were either undetectable or very low in abundance and quantification was unattainable. Additionally, feruloylquinic acid and caffeoyl-D-glucose could not be statistically analysed due to large number of missing values. There was zero abundance of isorhamnetin-3-rutinoside and isorhamnetin-3-glucoside in both stocks sampled.

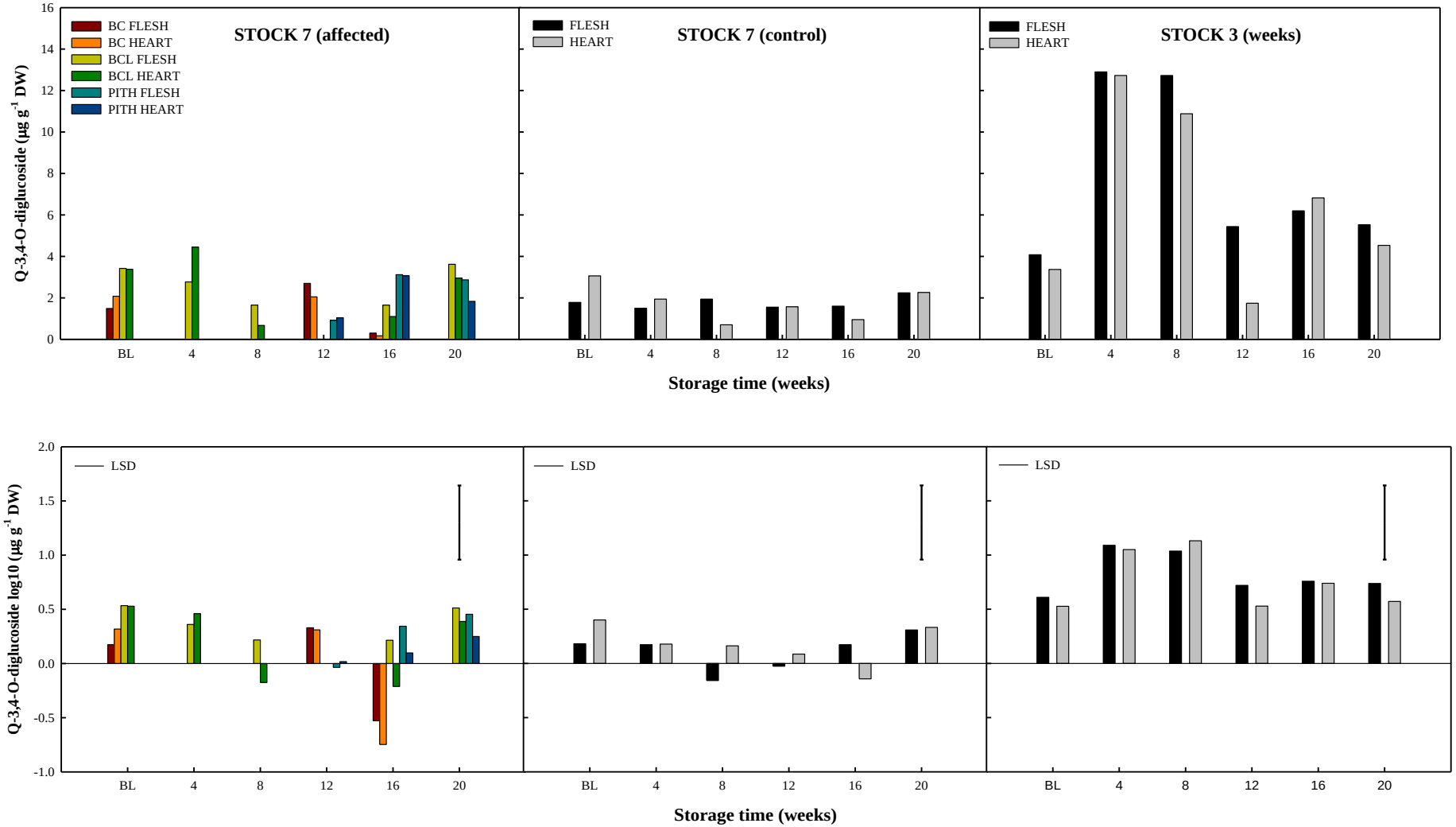


Figure 5.8 Quercetin-3,4-O-diglucoside unlogged and logged10 concentrations (µg g^{-1} DW) in flesh and heart tissue of potato tubers cv. Maris Piper stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) after baseline and storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks. General LSD is shown (Appendix C; Table 6.7 – 6.8).

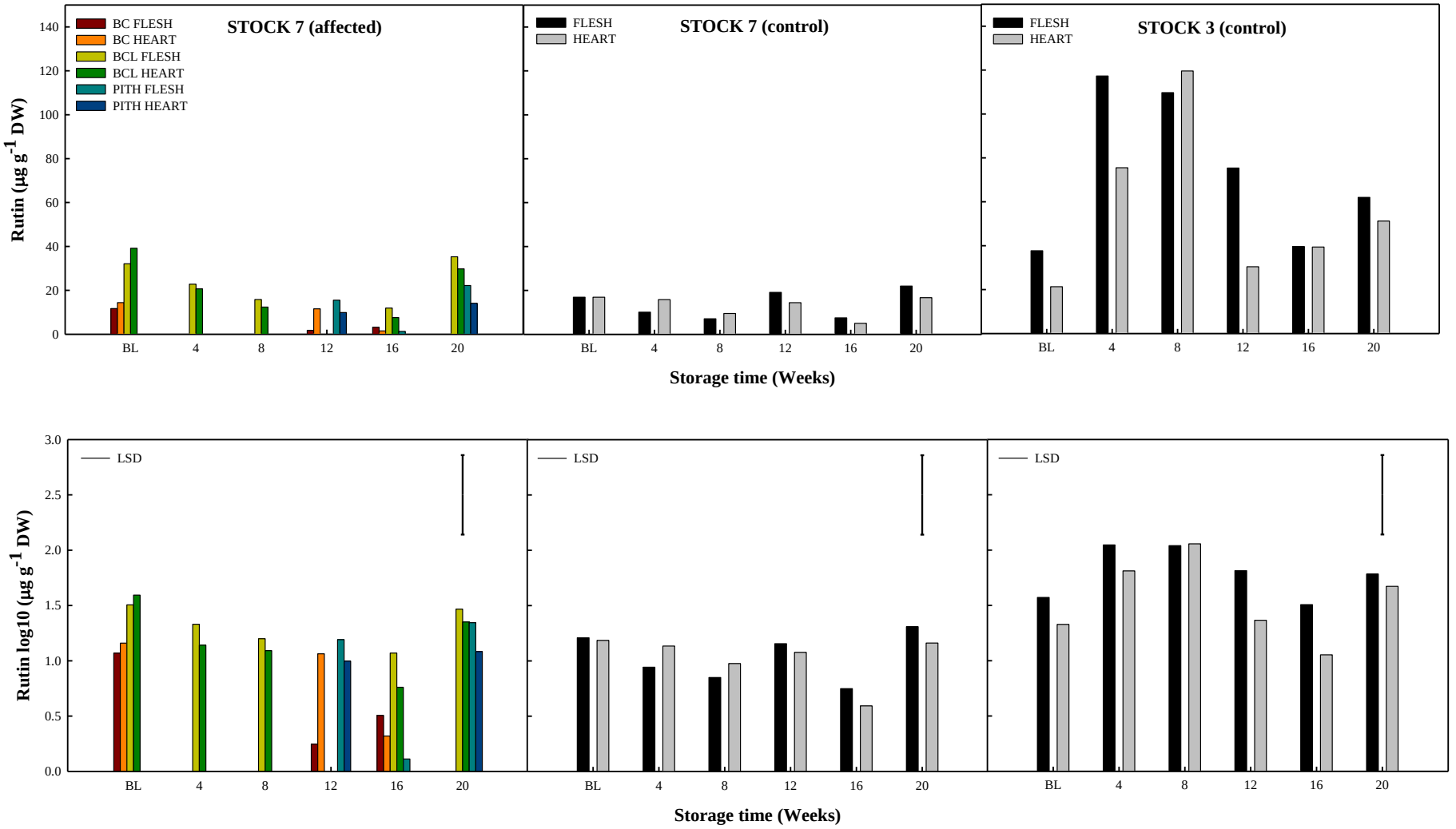


Figure 5.9 Rutin unlogged and logged10 (µg g^{-1} DW) in flesh and heart tissue of potato tubers cv. Maris Piper stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) after baseline and storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks. General LSD is shown (Appendix C; Table 6.9 – 6.10).

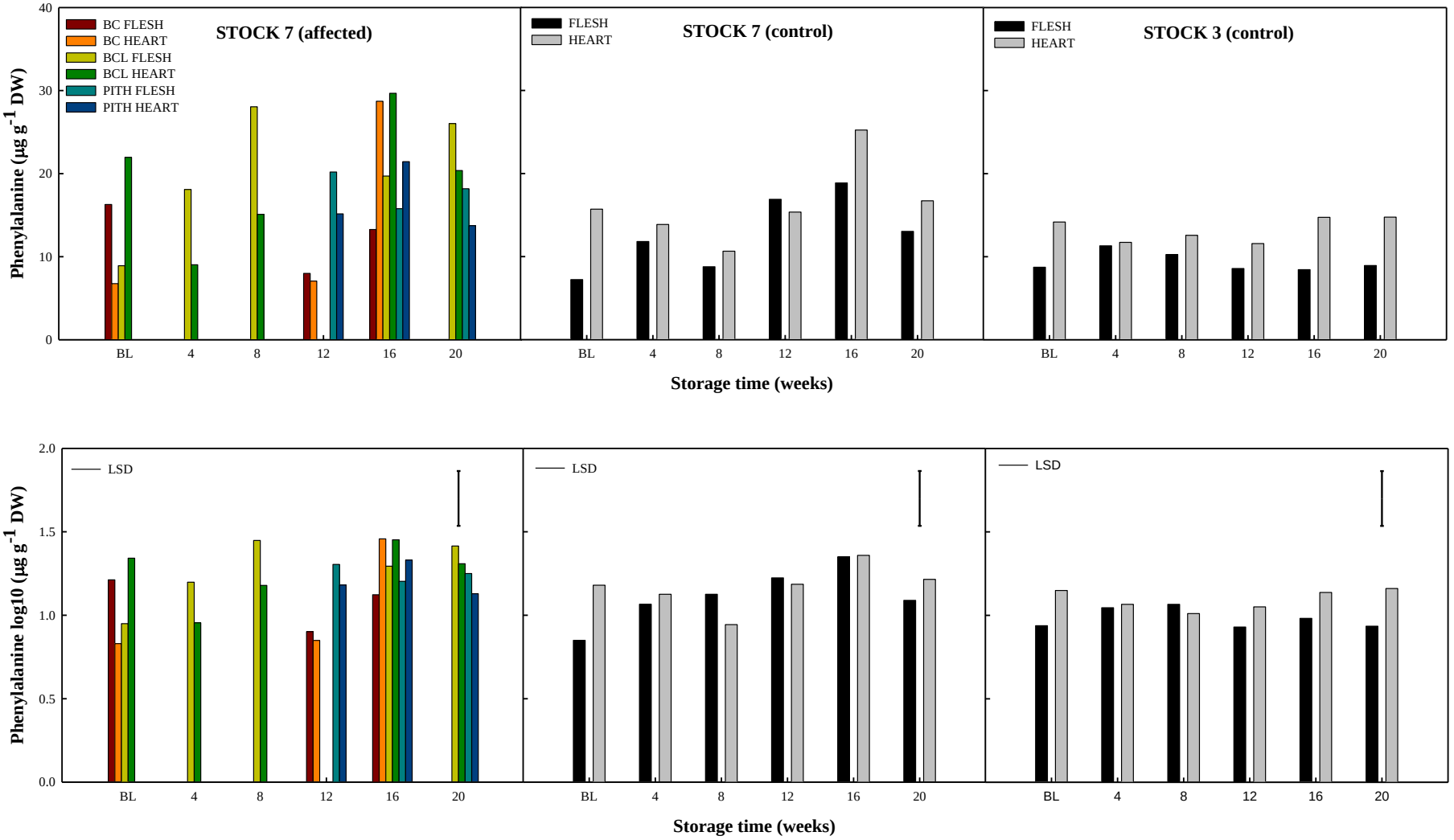


Figure 5.10 Phenylalanine unlogged and logged10 concentrations (µg g^{-1} DW) in flesh and heart tissue of potato tubers cv. Maris Piper stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) after baseline (0 weeks) and storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks. General LSD is shown (Appendix C; Table 6.11 – 6.12).

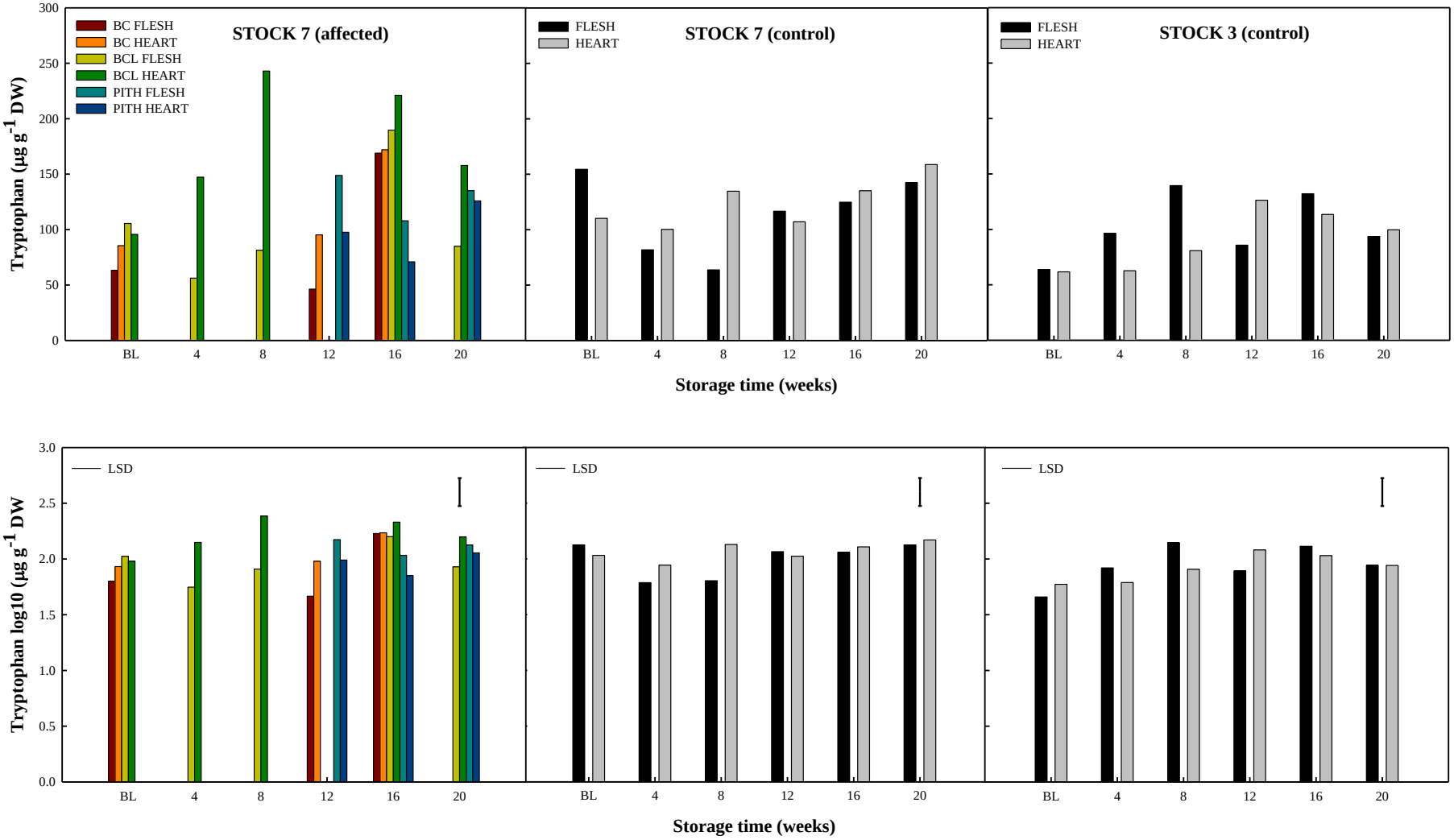


Figure 5.11 Tryptophan unlogged and logged10 concentrations (µg g^{-1} DW) in flesh and heart tissue of potato tubers cv. Maris Piper stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) after baseline (0 weeks) and storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks. General LSD is shown (Appendix C; Table 6.13 – 6.14).

5.3.3.3. Multivariate analysis

Sugar and phenolic compounds were quantified in 96 samples (flesh and heart tuber tissues) in total (section 5.3.3.1 and 5.3.3.2.). Three sugar variables (fructose, glucose and sucrose) combined with seven phenolic compounds (chlorogenic acid, neo-chlorogenic acid, crypto-chlorogenic acid, quercetin-3,4-O-diglucoside, rutin, tryptophan and phenylalanine) were used as analytical data for PCA. PCA was carried out in order to further visualize the accumulation of sugar and phenolic compounds in flesh and heart tissue samples of two stocks [stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH)]. Both stocks were stored at 20^{o}C in air only and initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks of storage (sampling points). There was no initial storage temperature at baseline. However, PCA biplots were formed over storage time and tuber tissues labelled according to stock [1= stock 7(susceptible to BH) and 2= stock 3 (non-susceptible to BH)] corresponded with tissue discoloration [*viz.* pith, brown centre light (BCL) and brown centre (BC)]. Control samples of stock 7 were labelled as 'C' meaning control= no discoloration and even though no tissue discoloration was indicated in flesh samples of stock 7 labelling was carried out according to tissue discoloration just to clarify the affected tuber from which the samples were derived. Samples of stock 3 showing no tissue discoloration labelled as 2 (Fig. 5.12a and b).

The PCA on the flesh tissue data showed a clear separation of the stocks on PC1 (captured 93.62% of the variance) and PC2 (captured 3.49% of the variance) capturing almost 97% of the variance in total (Fig 5.12a). Stock 7 (susceptible to BH) was clearly clustered according to sugars. On the other hand, neo- and crypto-chlorogenic acids, rutin and quercetin-3,4-O-diglucoside contributed for separating stock 3 (Fig. 5.12a).

The PCA on the heart tissue data showed a clear separation of the stocks on PC1 capturing 97.36% of the variance and PC2 only captured a further 1.52% of the variance

(98.8% of the variance in total) (Fig. 5.12b). A clear separation for those heart samples of stock 3 (non-susceptible to BH) was observed which according to the biplot corresponded with higher rutin and quercetin-3,4-O-diglucoside whereas phenylalanine was a less important variable separating stock 3 from stock 7. On the other hand, a separation between control and discoloured heart samples of stock 7 was not clearly observed. A few control samples were grouped according to crypto- and chlorogenic acid and reducing sugars along with some pith samples. BCL heart samples were randomly clustered according to tryptophan and sucrose (Fig. 5.12b).

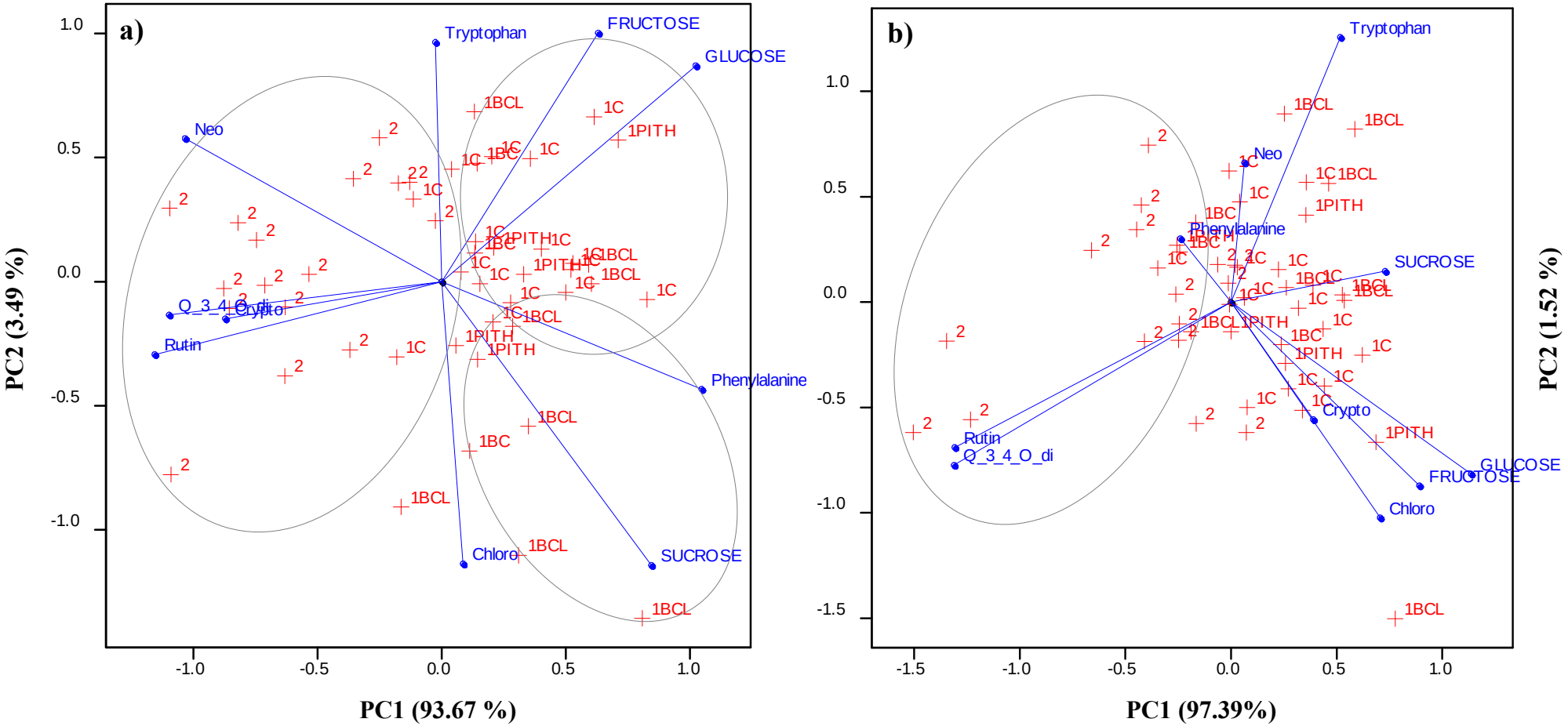


Figure 5.12 General PCA biplot of flesh (a) and heart (b) tuber tissue labelled by stock [1= stock 7 (susceptible to BH) and 2= stock 3 (non-susceptible to BH) corresponded with or without tissue discoloration [C= non-affected (control), PITH, brown centre light (BCL) and brown centre (BC)]. Tubers were stored at 20^{o}C in air and initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks of storage. Biplots formed over storage time.

B) Experiment 2

5.3.4. Respiration rate

Tuber respiration rate was recorded for potatoes cv. Maris Piper stock 7, stock 12 (susceptible to BH stocks) and stock 3 (non-susceptible to BH) after baseline (day 0) in air only and after 3, 7, 10 and 14 days of storage at 20^{o}C in four different gas combinations (*viz.* A = 21% O_2, B = 10% CO_2, C = 10% O_2 and D = 5% O_2). Tubers have initially been stored at 1.5^{o}C for ca. 8 months. However, efflux of CO_2 was included in respiration rate for those tubers treated with 10% CO_2. Statistical analysis was performed with GenStat 16th Edition (VSN International Ltd., Herts., UK) using ANOVA in order to estimate the interactions between factors on CO_2 production of potato tubers. These factors included were the tuber stock [stock 7(susceptible to BH), stock 12 (susceptible to BH) and stock 3 (non-susceptible to BH)], sampling days (day 0, 3, 7, 10 and 14) and various gas combinations (*viz.* A = 21% O_2, B = 10% CO_2, C = 10% O_2 and D = 5% O_2).

In general, the highest and the lowest respiration rates were recorded after storage in 10% CO_2 and 5% O_2 respectively along storage time (3 to 14 days). On sampling day 0 (baseline), respiration rate was ca. two times higher for those tubers of stock 12 (susceptible to BH) when compared to stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) with concentrations of 9.29, 5.2 and 6.14 ml CO_2 kg h^{-1} respectively. Respiration rate was relatively constant for all three stocks after 3, 7 and 10 days of storage in air only (A = 21 % O_2) and remained constant for stock 3 (non-susceptible to BH) until day 14 (Fig. 5.17a). However, a significant increase in CO_2 production was observed for those tubers of both susceptible stocks (12 and 7) on sampling day 14 (Fig. 5.17a).

When tubers were held under 10% CO_2, respiration rate did not change on sampling day 3 for both susceptible stocks (12 and 7) compared to those rates after storage in air (21% O_2),

but was significantly increased on day 7, remained constant on day 10 and then increased again on day 14. However, respiration rate recorded on sampling day 10 did not change for stock 7 after 10% CO_2 compared to 21% O_2 (Fig. 5.17a,b). The highest CO_2 rates at that storage condition (10% CO_2) were recorded by stock 3 (non-susceptible to BH). The respiration rate of stock 3 remained constant from day 3 to 10 and then significantly increased on day 14 (17.49 ml CO_2 kg h^{-1}) (Fig. 5.17b).

A 'W' shaped pattern was followed by both susceptible stocks (12 and 7) after storage in either 10 or 5% O_2 where respiration rate was decreased on sampling day 3 and 10 and increased on day 7 and 14 (Fig. 5.17c,d). However, that pattern was only followed by stock 3 (non-susceptible to BH) when stored in 10% O_2 (Fig. 5.17c). A constant respiration rate remained for stock 3 after storage in 5% O_2 for 3, 7 and 10 days ranging from 3.25, 3.74 and 2.09 ml CO_2 kg h^{-1} respectively, but significantly increased on day 14 (5.19 ml CO_2 kg h^{-1}) (Fig. 5.17d). Also, on sampling day 14 respiration rate was about the same for stock 3 (non-susceptible to BH) after storage in either 5, 10 or 21% O_2 (Fig. 5.17a,c,d). Furthermore, on sampling day 7 when tubers stored in 10% O_2, respiration rate recorded by all stocks was significantly higher compared to storage at 21% O_2 and there were no changes in CO_2 production between 10% O_2 and 10% CO_2 storage for both susceptible stocks 7 and 12 (Fig. 5.17b,c).

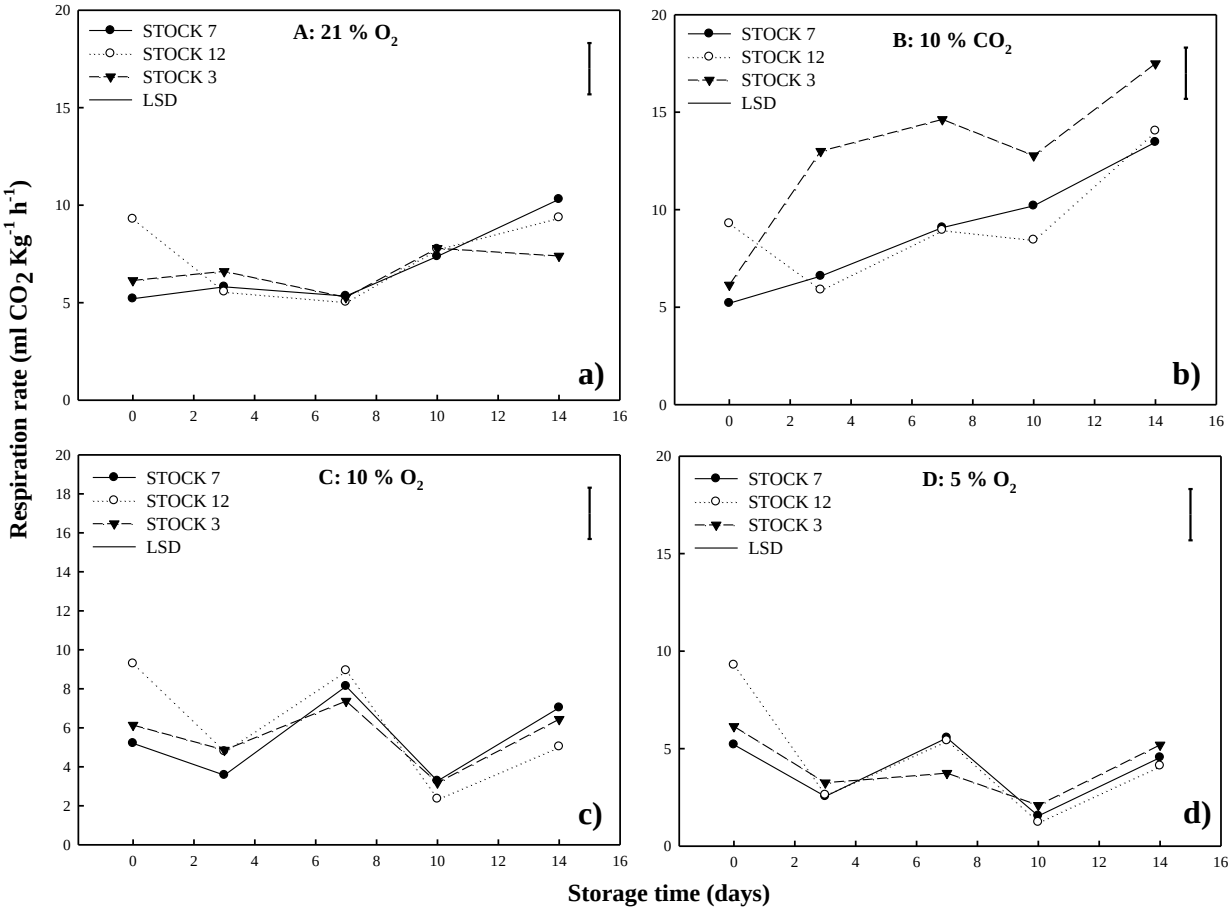


Figure 5.17 Respiration rate (ml CO_2 5 kg^{-1} h^{-1}) of potato cv. Maris Piper stock 7 (susceptible to BH), stock 12 (susceptible to BH) and stock 3 (non- susceptible to BH) recorded after baseline (day 0) and storage in four gas combinations (*viz.* A: 21% O_2, B: 10% CO_2, C: 10% O_2 and D: 5% O_2) at 20^{o}C on sampling days 3, 7, 10 and 14. Tubers were initially stored at 1.5^{o}C for ca. 8 months (Appendix C; Table 1).

5.3.5. The incidence of blackheart

The BH incidence of potato stocks after storage in four gas combinations (*viz.* A: 21% O_2, B: 10% CO_2, C: 10% O_2 and D: 5% O_2) at 20^{o}C was very low. Generally, BH incidence was greater for those tubers stored in 5% O_2. The total number of discoloured tubers of stock 7 (susceptible to BH) and stock 12 (susceptible to BH) are tabulated in Table 5.8 and Table 5.9 respectively. Due to very low BH incidence in both susceptible stocks (stock 7 and stock 12) through the storage period, Generalized Linear Models (GLM) analysis was used as

statistical tool using GenStat 16th Edition (VSN International Ltd., Herts., UK). GLM analysis extends the usual regression framework to cater for non-normal distributions incorporating a link function which defines the transformation required to make the model linear. In this data set a binomial distribution and logit function were used in order a linear model to be fitted.

GLM were performed in order to assess the incidence of BH of each susceptible stock separately after tuber storage in those four gas combinations where tubers sampled on sampling days 3, 7, 10 and 14. Baseline data was not included due to zero BH incidence observed. Regarding stock 7 (susceptible to BH) results showed that the *chi pr.* value was greater than 0.05 (chi pr. = 0.09) which means that neither gas combination nor sampling day explained a significance in BH incidence (Appendix C; Table 8) However, results for stock 12 (susceptible to BH) indicated a significance in BH incidence (chi pr. = 0.045) (Appendix C; Table 9) and that was due the highest indication of discoloured tubers of stock 12 on sampling day 10 (Table 5.9). Example figures of tubers showing tissue discolorations are presented in Appendix A; Figure 2.6 – 2.7).

Table 5.8 Total number of discoloured potato stocks cv. Maris Piper stock 7 (susceptible to BH) after baseline (day 0) and storage in four gas combinations (*viz.* A= 21% O_2, B= 10% CO_2, C= 10% O_2 and D= 5% O_2) at 20^{o}C on sampling days 3, 7, 10 and 14.

Tubers used/3 stocks	DAY	A= 21% O_2	B= 10% CO_2	C= 10% O_2	D= 5% O_2	TOTAL
27	0	0	0	0	0	**0**
108	3	0	2	1	1	**4**
108	7	1	0	0	2	**3**
108	10	0	1	0	3	**4**
108	14	0	0	0	1	**1**
	TOTAL	**1**	**3**	**1**	**7**	**12**

Table 5.9 Total number of discoloured potato stocks cv. Maris Piper stock 12 (susceptible to BH) after baseline (day 0) and storage in four gas combinations (*viz.* A: 21% O_2, B: 10% CO_2, C: 10% O_2 and D: 5% O_2) at 20^{o}C on sampling days 3, 7, 10 and 14.

Tubers used/3 stocks	DAY	A: 21% O_2	B: 10% CO_2	C: 10% O_2	D: 5% O_2	**TOTAL**
27	0	0	0	0	0	**0**
108	3	0	1	0	1	**2**
108	7	0	0	0	1	**1**
108	10	0	1	3	2	**6**
108	14	0	1	0	0	**1**
	TOTAL	**0**	**3**	**3**	**4**	**10**

5.4. Discussion

5.4.1. The effect of storage temperature and shelf-life conditions on the respiration rate of potato stocks with different susceptibility to BH

Results from the 1st experiment revealed that respiration rate recorded at baseline on sampling day 0 which was the arrival day of the potato stocks in the lab was ca. 8-9 ml CO_2 kg^{-1} h^{-1}. However, when tuber sub-samples placed in chambers with regular air circulation and sampled at 20^{o}C respiration rate significantly decreased to 4.6-5.3 ml CO_2 kg^{-1} h^{-1} showing no significant differences between those stocks with different susceptibility to BH on both sampling days. That decrease in respiration rate might possibly have occurred because on sampling day 0 namely the arrival day potato tubers experienced a variation of temperature changes until the time of respiration rate measurement, so when sampled on day 7 respiration rates had already been stabilized (Craft, 1963; Schippers, 1977a). Also, it was observed that once tubers stored at initial cold storage temperature (1.5^{o}C), stock 3 (non-susceptible to BH) had the greater respiration rate on each sampling day 0 at 20^{o}C after 4, 8

and 12 weeks of cold storage compared to both susceptible stocks whereas their respiration rates showing no significant differences. In comparison with year 1 analysis, those CO_2 rates recorded of all stocks on sampling day 0 at 20^{o}C were a bit higher than those of year 1 when tubers sampled on day 0 in air and have been initially stored at 1.5^{o}C. On the other hand, respiration rates recorded on sampling day 7 were lower compared to those CO_2 rates of year 1 at the same storage conditions. Also, in year 1 it was shown that respiration rate was increasing from day 0 to 3 and then slightly decreasing from day 3 to 7; however, a similar pattern could not be observed in year 2 as respiration rate recorded on two sampling days (*viz.* 0 and 7). It should be noted that those CO_2 rates in year 2 were recorded individually in a jar for each tuber whereas in year 1 three tubers were placed in a jar for respiration rate measurements (see Chapter 3; Section 3.4), so that might explain those differences in respiration rate and also the fact that all stocks analysed have been grown in different locations and under different growth conditions in both year analysis might have an impact on their physiology.

In the second experiment of year 2, potato stocks have already been initially stored at 1.5^{o}C and when sampled on day 0 considered as baseline stock 12 (susceptible to BH) showed greater respiration rate compared to stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH). When tuber subsamples were placed in chambers with various gas combinations (*viz.* A: 21% O_2, B: 10% CO_2, C: 10% O_2 and D: 5% O_2) and sampled on days 3, 7, 10 and 14 respiration rate varied, but significant differences between those stocks analysed were more evident under storage at 10% CO_2 where stock 3 scored the highest CO_2 rates compared to both susceptible stocks (7 and 12). However, the CO_2 absorption of both susceptible stocks held under 10% CO_2 was lower on sampling day 3 where respiration rates were lower for stock 7 and stock 12 compared to stock 3. Then, from day 3 to 7 there was an increase in respiration rate for both susceptible stocks which remained constant till day 10

and increased again on sampling day 14 reaching those CO_2 rates of stock 3 (non-susceptible to BH). Those findings show the differences in CO_2 tolerance between those stocks analysed (Mathooko, 1996; Kader, 2003). Also, those rates recorded by stock 3 were similar to those recorded in year 1 of tubers held under 10% CO_2 and previously been stored at 1.5^{o}C. Although respiration rates were lower after storage in 5% O_2 as it was expected, however, there was a variation in CO_2 rates produced by all stocks after storage in 10 or 5% O_2 including similarities and differences compared to the other gas combinations applied.

Those findings indicate no relation in respiration rate and potato stock susceptibility to BH; firstly because the non-susceptible stock 3 showed greater CO_2 production when held under 10% CO_2 storage in which is believed that tuber tissue discoloration being stimulated and secondly there were no significant differences in respiration rate between affected and non-affected samples of those susceptible stocks analysed (7 and 12) after 'Two-sample' t-test performances.

5.4.2. The effect of storage temperature and shelf-life conditions on the BH incidence of potato stocks with different susceptibility to blackheart disorder

The incidence of BH in both experiments was quite low proportionally to the total number of tubers used. In the 1st experiment, BH incidence was initially indicated at baseline (December 2012) and peaked after a few months (March and May 2013); however, no significant differences in BH incidence were shown over storage time and yet between those susceptible to BH stocks 7 and 12. Brown tissue discolorations (BC, brown centre; BCL, brown centre light and pith) were only indicated in 16 out of 108 tubers of stock 7 (susceptible to BH) and 12 out of 108 tubers of stock 12 (susceptible to BH) randomly on sampling days 0 an 7 over storage time. It could be said that low BH incidence was due to

more normal storage conditions without O_2 exclusion, but on the other hand the BH incidence was equally low in the 2nd experiment where potato tubers held under various gas combinations (*viz.* A= 21% O_2, B= 10% CO_2, C= 10% O_2 and D= 5% O_2) and only 12 out of 153 tubers of stock 7 and 10 out of 153 tubers of stock 12 showed indications of tissue discoloration. In contrast with the year 1, in this year 2 dark brown to black discolorations were not observed in any of the experiments conducted and also in year 1 no discolorations were shown at baseline. This difference in BH incidence between year 1 and year 2 might be explained due to different growing seasons and growth conditions that all potato stocks analysed have been grown at (Davies, 1998).

Furthermore, the possible effect of tuber size and weight on BH incidence was also examined comparing affected and non-affected tubers of both susceptible stocks 7 and 12. In general, tubers derived from all three potato stocks and used in both year 2 experiments had similar tuber weight and tuber size. In the 1st experiment tuber weight and size did not have an effect on BH incidence of stock 7 over storage time. However, after 16 weeks of storage tuber weight and BH incidence were weakly positive correlated for those tubers of stock 12 where affected tubers had greater weight compared to those showing no affection, but there were no correlations regarding the tuber length and diameter and also no further significant effects on BH incidence according to tuber weight and size were shown for stock 12 over storage time. Thus, according to those findings and due to the low indication of tissue discoloration it cannot be concluded that tuber weight and size have an effect on the incidence of BH.

5.4.3. The effect of storage temperature and shelf-life conditions on the sugar content of potato stocks with different susceptibility to blackheart disorder

PCA results revealed that potato stocks with different susceptibility to BH could be well grouped according to sugar and phenolic content over storage time. Generally, stock 7 (susceptible to BH) contained higher sugar content. On the other hand both flavonoid compounds (rutin and quercetin-3,4-O-diglucoside) were those variables separating stock 3 (non-susceptible to BH). Moreover, chlorogenic acid isomers were highly contained in those flesh samples of stock 3 and chlorogenic and crypto-chlorogenic acids in heart samples of stock 7. It should be reminded that those flesh samples of stock 7 that were labelled according to tissue discoloration [viz. pith, brown centre light (BCL) and brown centre (BC)] they were not discoloured but that labelling was carried out in order to just clarify the affected tuber from which those flesh samples derived.

In general, reducing sugar content was ca. 2-fold higher in heart than flesh tissue samples of both stocks when measured at 20^{o}C. Fructose accumulation was mainly increased after 4 weeks of storage where there was no initial storage temperature, but glucose was already highly concentrated in those discoloured heart samples of stock 7 and control heart samples of stock 3. Reducing sugar content of stock 7 (susceptible to BH) was similar to that of stock 23 (susceptible to BH) measured in year 1. However, in year 2 fructose and glucose did not increase during storage time. Sucrose content was significantly higher in discoloured samples of stock 7 after baseline and 4 and 8 weeks of storage, but there were no differences between discoloured and control samples after 12, 16 and 20 weeks. Also, changes in sucrose content of stock 3 (non-susceptible to BH) were not shown over storage time, but it was significantly lower compared to stock 7. Compared to year 1, sucrose content was slightly higher in year 2 and yet higher in discoloured samples after baseline, 4 and 8 weeks of

storage. In year 1, sugar content quantification in flesh and heart tissue was carried out regardless tissue discoloration, but according to PCA biplots it was shown that discoloured samples contained higher sugar content. Furthermore, in year 2 the fact that reducing sugar accumulation was similar between discoloured and control samples of stock 7, it cannot really highlight that sugars accumulate more due to tissue discoloration as it was shown in year 1 and that might happen due to low BH incidence indicated in this study. On the other hand, it seems that stock 7 is more susceptible to low storage temperature compared to stock 3 and that was more evident due to high sucrose content observed over storage time.

5.4.4. The effect of storage temperature and shelf-life conditions on the phenolic content of potato stocks with different susceptibility to blackheart disorder

In terms of phenolic content, chlorogenic acid was the major phenolic acid quantified in flesh and heart samples of both stocks analysed, accumulated more in stock 7 (susceptible to BH) and yet significantly higher in heart samples of stock 7 compared to stock 3 (non-susceptible to BH). In addition, heart chlorogenic acid content of stock 3 was significantly lower almost over storage time. However, flesh chlorogenic acid accumulation varied between the stocks and that might happen because none of those flesh samples showed any tissue discoloration. It is worth noting though that similar trend in chlorogenic acid content was also shown in year 1 analysis where it was found being accumulated in those heart samples of stock 23 (susceptible to BH) which had greater susceptibility to BH. On the other hand, its content was much higher in this study as samples of stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) contained ca. 2-9 and 2-3 times higher chlorogenic acid compared to stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) in year 1 respectively. It should be noted that all stocks analysed in both years have been grown at different locations and growing conditions and seasons, so that might have an impact on PAL

enzyme activity which is the precursor enzyme of the phenylpropanoid metabolism (Hamouz *et al.*, 2010; Ieri *et al.*, 2011; Adamo *et al.*, 2012; Payyavula *et al.*, 2012). Chlorogenic acid isomers neo-(3-O-caffeoylquinic acid) and crypto-chlorogenic acid (4-O-caffeoylquinic acid) showed greater accumulation in flesh samples of stock 3 (non-susceptible to BH) compared to stock 7 (susceptible to BH) and yet crypto-chlorogenic acid tended to be more accumulated in those heart samples of stock 7 regardless tissue discoloration. Interestingly, a similar trend in both isomers accumulation with similar concentrations was shown in flesh samples of stock 12 (non-susceptible to BH) in year 1.

Among those amino acids examined phenylalanine which is the precursor for the biosynthesis of phenylpropanoid compounds varied in content between stock 7 and stock 3. Similarly for tryptophan a distinct trend was not shown, but heart samples of stock 7 showing light brown discoloration (BCL) tended to accumulate more tryptophan. Unfortunately, quantification of tyrosine was not feasible due to large number of missing values. From those findings, a full conclusion on the amino acid accumulation cannot be made.

In order to further identify more phenolic compounds that possibly be related with tissue discoloration and/or potato stock susceptibility to BH, both flavonols rutin and quercetin-3,4-O-diglucoside were quantified. Rutin is a flavonol with pathogen defence potential and commonly shared in potatoes (Kreft *et al.*, 1999; Kroner *et al.*, 2012). Further, quercetin-3,4-O-diglucoside has previously been reported in onions (Takahama *et al.*, 2004). Both flavonols showed greater accumulation in both tissue samples of stock 3 (non-susceptible to BH) and that was more evident after 4 weeks of storage where initial storage temperature (1.5^{o}C) occurred. That increase in both flavonols could be result of cold-stress response as flavonoid pathway may be stimulated under low storage temperature (Cheynier *et al.*, 2009). In general, rutin content in potatoes may range from 0 – 400 μg g^{-1} DW (Evers and Deußer, 2009) and up to 800 μg g^{-1} DW in potato cv. Desiree (Lukaszewicz *et al.*, 2004). In this

study, rutin in stock 3 ranged from 20 to 120 μg g^{-1} DW whereas samples of stock 7 accumulated less than 40 μg g^{-1} DW.

5.5. Conclusions

To conclude, a relationship between respiration rate and BH incidence was not observed and that can be said because stock 3 (non-susceptible to BH) showed greater CO_2 levels in general and yet when held under 10% CO_2 storage in which is believed that tuber tissue discoloration might be stimulated. Therefore, there were no significant differences in respiration rate between affected and non-affected samples of those susceptible stocks analysed (7 and 12) after 'Two-sample't-test performances. Furthermore, the incidence of BH was low proportionally to the number of tubers used; however brown tissue discolorations were observed in heart samples of both susceptible stocks (stock 7 and stock 12) but a significant difference in their susceptibility was not shown. It could be said that the storage conditions in the 1st experiment where there was no exclusion of O_2 contributed to low incidence of BH; however when tubers stored under various gas combinations (*viz.* A: 21% O_2, B: 10% CO_2, C: 10% O_2 and D: 5% O_2) during the 2nd experiment BH incidence was equally low and tissue discolorations were observed under storage at 21% O_2 as well as it was shown in year 1. It is still unclear whether those brown tissue discolorations were developed due to low initial storage temperature (1.5°C), as indications were also shown at baseline. Biochemical analysis was carried out only using flesh and heart tissue samples of stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH). Similar trends to those of year 1 analysis in sugar and phenolic content were observed. Neo- and crypto-chlorogenic acid along with rutin and quercetin-3,4-O-diglucoside tended to be more accumulated in stock 3 whereas chlorogenic acid and crypto-chlorogenic acid along with sugars were more accumulated in stock 7. However, the results varied and a full conclusion cannot be made.

Also the fact that chlorogenic acid was highly accumulated in both control and discoloured samples of stock 7 makes unclear whether is a marker for susceptibility.

CHAPTER SIX:
Untargeted metabolomics analysis on potato stocks with different susceptibility to blackheart disorder

6.1. Introduction

Nowadays, metabolomics studies or '–omics' approaches is a major path evolving in food science and other scientific fields aiming to comprehensively profile and identify as larger a number as of possible metabolites providing a better understanding of how an organism responds or a tissue functions (Dunn and Ellis, 2005; Vorst *et al.*, 2005; Genga *et al.*, 2008). Metabolomics analysis can be performed using targeted analysis which centred on selected classes of metabolites (e.g. amino acids, organic acids, glycoalkaloids etc.) or un-targeted analysis in order to detect and identify the total content of a sample providing information on which metabolites are the most highly concentrated (de Voss *et al.*, 2007; Patti *et al.*, 2012). Metabolites are low molecular weight molecules present in a cell and are required for metabolic reactions and the collection of these metabolites is called the metabolome and it has been estimated that ca. 50.000 primary and secondary metabolites with various chemical structures present in plants and about 200.000 metabolites are predicted for the plant kingdom in total (Hall *et al.*, 2002; Allwood *et al.*, 2008; Genga *et al.*, 2008; Patti *et al.*, 2012). Due to extensive chemical diversity and complexity, a full profiling of the whole metabolome cannot be obtained and it is estimated that currently metabolite identification amounts ca. to 10% (Sumner *et al.*, 2002; Alliferis and Jabaji, 2012). However, several analytical techniques such as Mass Specrtometry (MS), Nuclear Magnetic Resonance (NMR), Fourier Transform Infared (FT-IR), have been applied in metabolomics analysis with Mass Spectrometry (MS) coupled with Gas Chromatography (GS-MS) or Liquid

Chromatography considered as the most applied technology for metabolomics analysis to date (Vorst *et al.*, 2005; Hall, 2005; Shepherd *et al.*, 2007).

In the last two decades, the potato crop has been extensively selected as a model plant for metabolomics analysis using a range of MS tools with GS-MS being the most utilised. Previous publications mainly referred to metabolite profiling for compositional changes and comparison among conventional and genetically modified (GM) potatoes (Roessner *et al.*, 2001; Deferenz *et al.*, 2004; Catchpole *et al.*, 2005; Lehesranta *et al.*, 2005) or phytochemical diversities in various Solanum species (Dobson *et al.*, 2000, 2007, 2008; Davies, 2007; Uri *et al.*, 2014). Therefore, a small number of metabolomic studies on potato developmental processes, wounding metabolism and pathogen invasion/response, have also been reported (Yang *et al.*, 2007; Shepherd *et al.*, 2010; Aliferis and Jabaji, 2012; Pushpa *et al.*, 2013).

Since the main object of metabolomics is to provide a comprehensive and thus more detailed molecular understanding of metabolite functions within a plant tissue by investigating the metabolome, it would be interesting to further identify as many as possible metabolites that might be related with tuber tissue discoloration and which may possibly be involved in the development of blackheart disorder (BH) in potato tubers. An untargeted metabolomic based approach on tuber tissues (flesh and heart) derived from potato cv. Maris Piper stocks with different susceptibility to BH was evaluated using a Liquid Chromatography (LC) coupled with an Accurate-Mass Quadrupole Time of Flight (Q-ToF) mass spectrometer (MS) instrument as a high-resolution tool. The purpose of this study was to find metabolic differences between discoloured and non-discoloured samples and morover the metabolic differences in potato stock susceptibility to BH. Although, there is some literature on the biochemistry of BH disorder in potatoes (Bartholomew, 1914; Reeve, 1968),

these references are old. This does not been they are invalid but that with modern analytical tools a greater understanding of the BH should be possible.

6.2. Materials and methods

Materials and methods for Chapter 6 were described in Chapter 3; Section 3.6.

6.3. Results

A) Year 1 (2011-2012)

6.3.1. Metabolomic differences between affected and non-affected potato tuber tissues

In both 'experiments A and B' (in negative and positive ion mode respectively), six samples per discoloration of stock 23 (susceptible to BH) were used for 'discoloration only' interpretation while those six samples halved to three for 'tissue vs. discoloration' interpretation (n = 3 biological samples / tissue / discoloration) (Fig. 6.1).

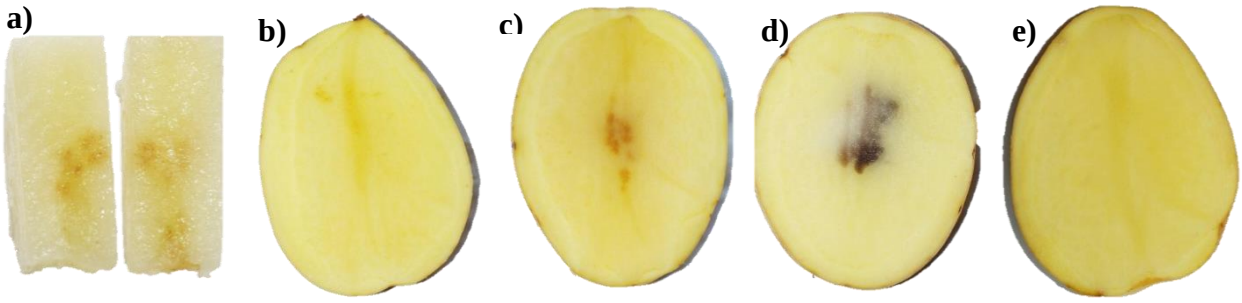


Figure 6.1 Example of tuber tissue discoloration: a) BCL: brown centre light (heart part), b) pith, c) BC: brown centre, d) BH: dark brown to black and e) C: control – no affection.

Negative ionization mode

Generally, 374 metabolites in total were identified as known and unknown in negative mode. In terms of the general interpretation 'discoloration only' (regardless the tissue) quality control on samples showed that 97 reproducible metabolites remained after filtering by frequency. Further filtering followed using filter by sample variation (10%) and 30 out of 97 metabolites left to be interpreted. PCA followed where all the possible principal components were calculated and visually represented per discoloration condition coloured-coded in a 3D scatter plot (Fig. 6.2). The PCA on the data showed a separation of tissue discoloration on x, y and z axis (capturing ca. 59% of the variance it total). A clear separation of BH discoloration was evident. Control and BCL discoloration were also well grouped; however, BC and pith discolorations were mixed with those conditions above mentioned (Fig. 6.2). Statistical analysis was performed on those 30 metabolites using Moderated t-test pairing each discoloration (BC, BCL, BH or pith) against control resulting in 19 known and unknown metabolites with a probability of $P < 0.05$ (95% that the metabolite was significant) (Appendix D; Table 2.1). Fold-change analysis was performed in order to look for significant differences between control compared with each discoloration and 13 out of 19 metabolites were either up or down regulated (Fig. 6.3). According to Figure 6.3, it was shown that 10 metabolites including two unsaturated hydroxy fatty acids, purine and pyrimidine related metabolites were all down regulated in discolorations when compared with the control. Also, an unknown compound with formula [C_7 H_4 N_4 O_2] was up regulated in BC, BCL and pith but not in BH discoloration. An up regulation of 5-Acetamidovalerate (which is involved in lysine degradation III pathway) was observed in BCL only when compared with the control (Fig. 6.3).

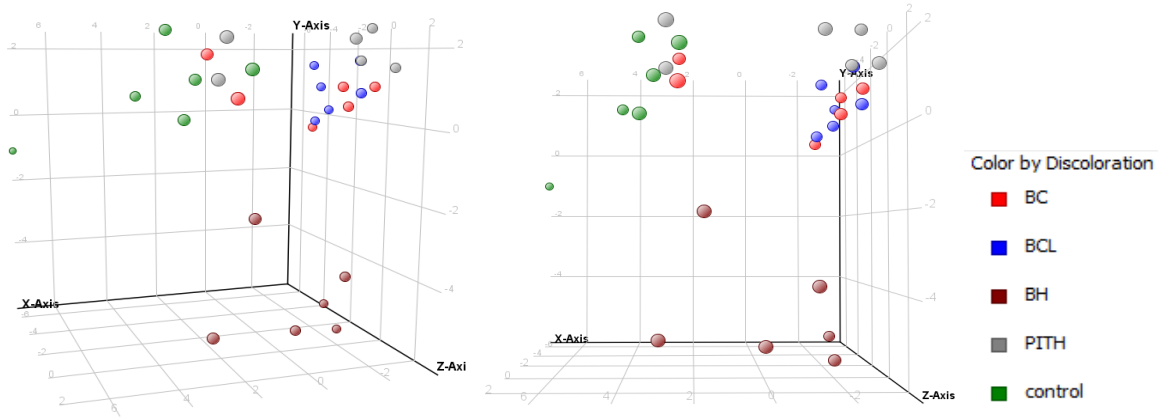


Figure 6.2 3D Principal Component Analysis scatter plot showing differences between tuber tissue discolorations and control of stock 23 (susceptible to BH) in negative mode (x= 27.1 %, y= 19.87%, z= 12.04%) (BC, brown centre; BCL, brown centre light; BH, dark brown to black discoloration).

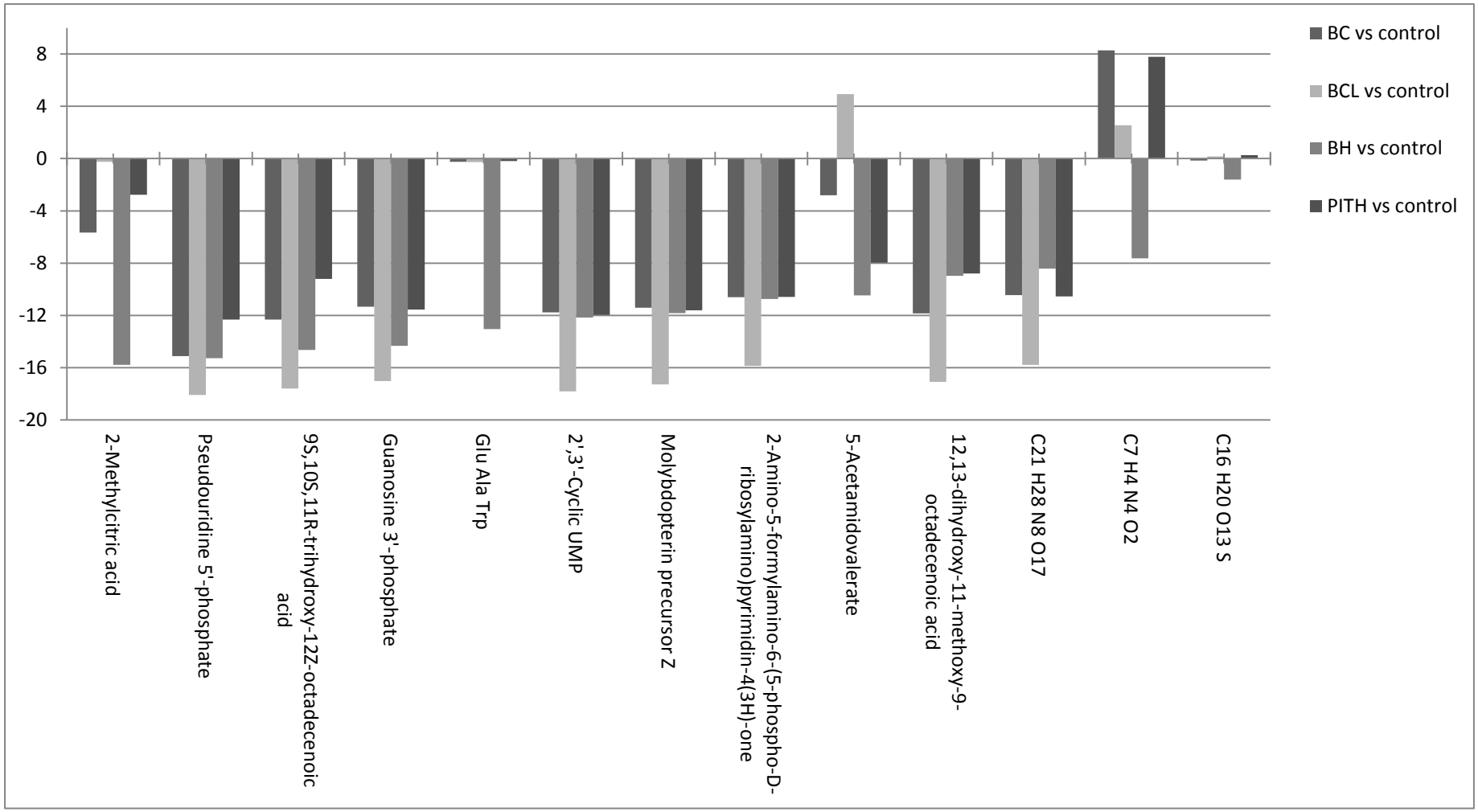


Figure 6.3 Fold change analysis results of 'discoloration only' interpretation in 'experiment A'. Metabolite regulation (log FC normalized) is shown in negative mode (BC, brown centre; BCL, brown centre light; BH, dark brown to black). (Appendix D; Table 2.2).

In terms of 'tissue vs. discoloration', interpreting flesh and heart samples with BH discoloration against flesh and heart control samples results showed 130 out of 374 reproducible metabolites left after frequency filtering and 65 out of 130 metabolites with 10% sample variation eventually remained. 3D PCA scatter plot showed one point per tissue sample colour-coded and shaped corresponding to BH discoloration or control. A clear separation on x, y and z axis (capturing ca. 70% of the variance in total) was observed (Fig. 6.4). Two-way ANOVA was performed on 65 metabolites resulting in 28 significant metabolites with a probability of $P < 0.05$ (95% that the metabolite is significant) (Appendix D; Table 2.3). Fold change analysis results revealed that 24 metabolites were up or down regulated. Two unknown compounds [C_8 H_7 N_3 O_5 and C_{21} H_{43} N_5 O_{14} S] were up-regulated in BH samples when compared with control samples. Furthermore, known metabolites as chlorogenic acid, 5-O-feruloylquinic acid, unsaturated fatty acids, glutathione oxidized and 4-Hydroxyphenylacetylglutamine (involved in tyrosine metabolism) showed a down regulation in BH samples (Fig. 6.5).

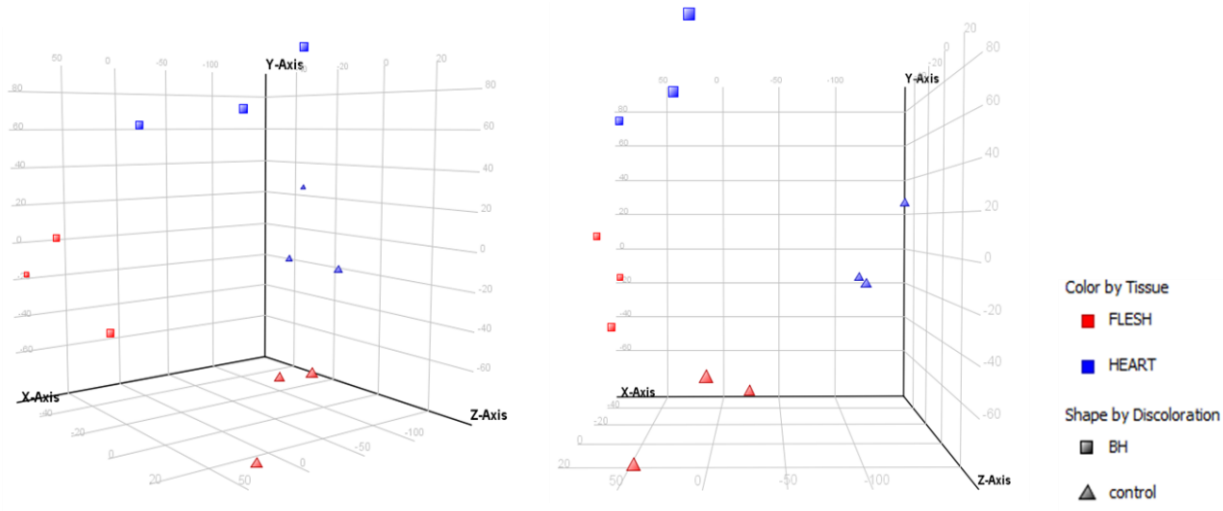


Figure 6.4 3D Principal Component Analysis scatter plot showing differences between BH (dark brown to black) and control samples of stock 23 (susceptible to BH) in negative mode (x= 35.39%, y= 22.81%, z= 12.22%).

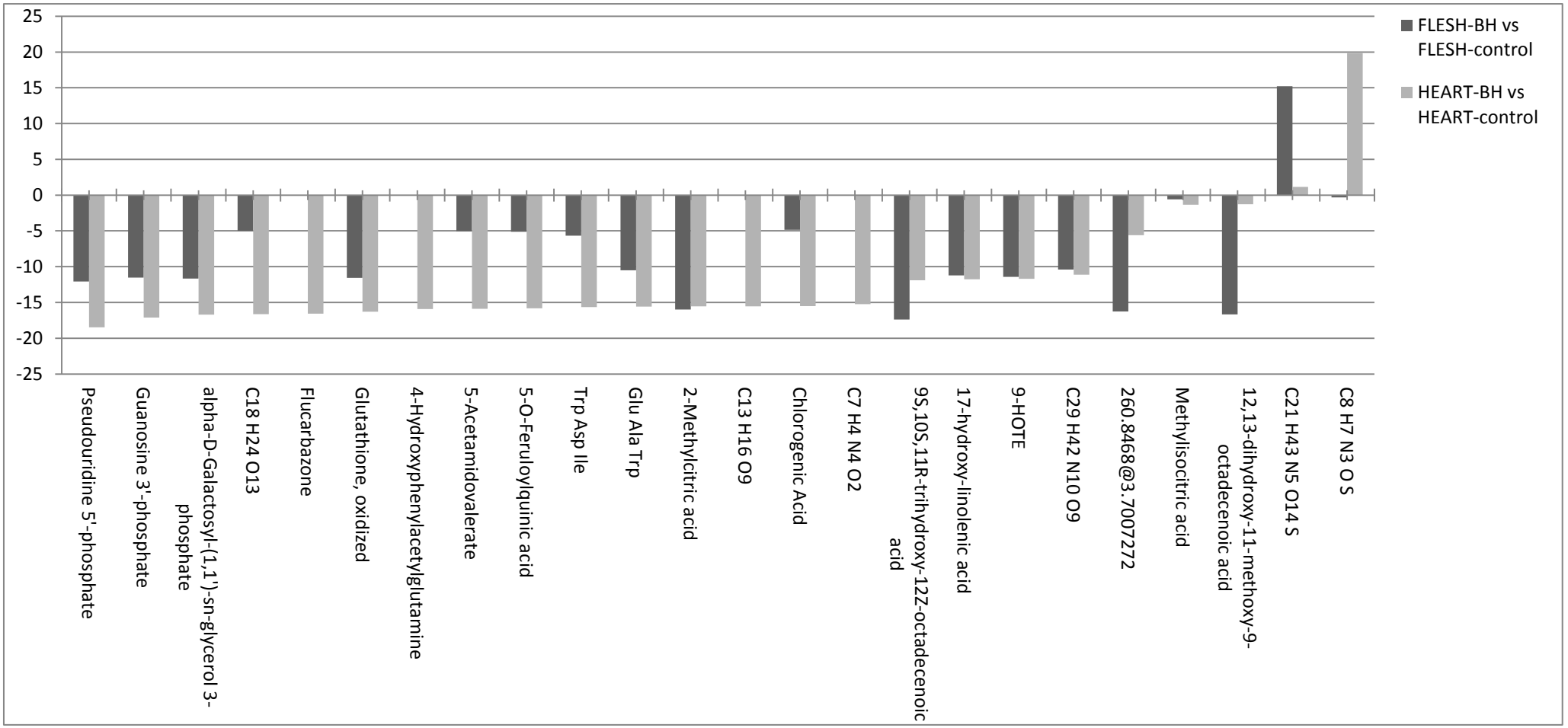


Figure 6.5 Fold change analysis results of 'tissue vs. discoloration' interpreting BH (dark brown to black) vs. control in 'experiment A'. Metabolite regulation (log FC normalized) is shown in negative mode (Appendix D; Table 2.4).

Interpretation between BC and control flesh and heart samples, 120 out of 374 metabolites remained after filter by frequency that were additionally filtered based on a coefficient of variation of less than 10% and 55 out of 120 entities left to be interpreted. According to the 3D PCA, ca. 64% of variance in total was explained on x, y and z axis and clear group among tissue and tissue discoloration were shown (Fig. 6.6). Two-way ANOVA pairing BC flesh against control flesh samples and BC heart against control heart resulted in 5 significant metabolites with a probability of $P < 0.05$ (Appendix D; Table 2.5). Fold change analysis results showed regulation of 4 metabolites where one unknown metabolite with formula (C_7 H_4 N_4 O_2) was up regulated in BC samples. 2,5 dioxopentanotate (involved in amino acid metabolism) and pseudouridine-5'phosphate (pyrimidine metabolism) showed down regulation (Fig. 6.7).

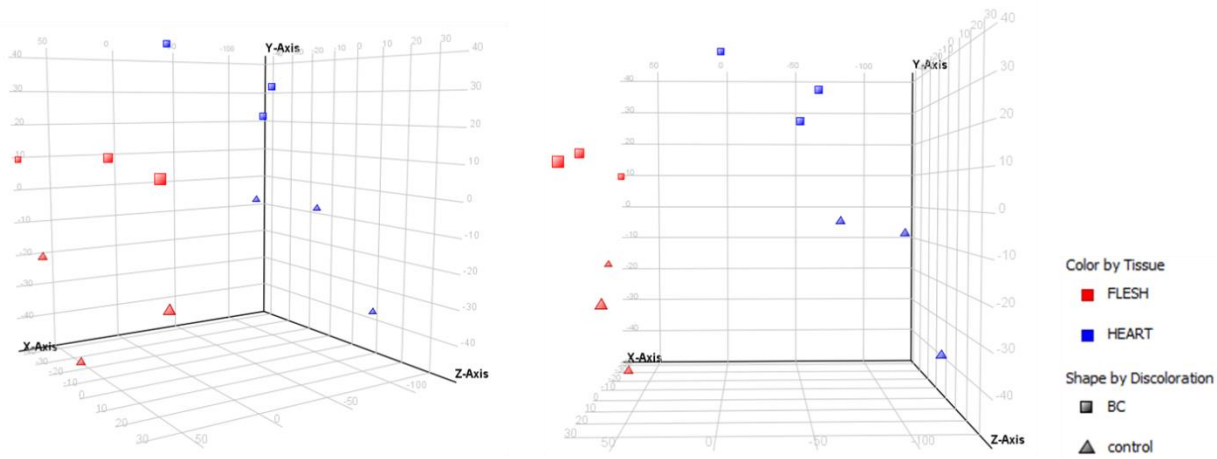


Figure 6.6 3D Principal Component Analysis scatter plot showing differences between BC (brown centre) and control tissue samples of stock 23 (susceptible to BH) in negative mode (x= 39.76%, y= 13.44%, z= 11.29%).

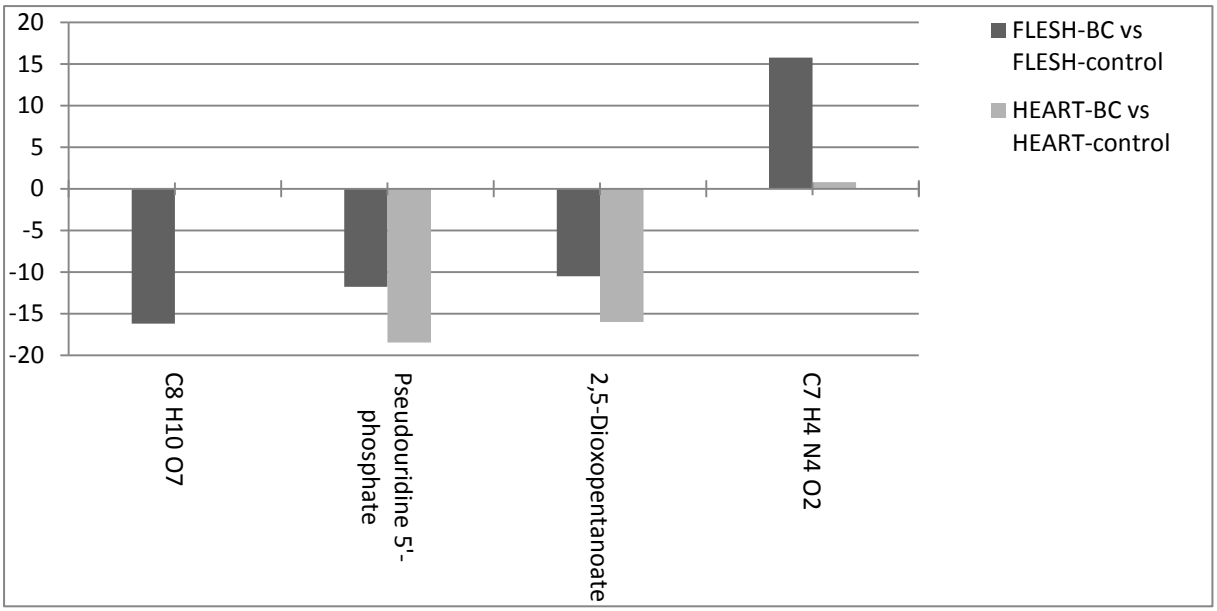


Figure 6.7 Fold change analysis results of 'tissue vs. discoloration' interpreting BC (brown centre) vs. control in 'experiment A'. Metabolite regulation (log FC normalized) is shown in negative mode (Appendix D; Table 2.6).

BCL against control interpretation showed that 122 out of 374 and 54 out of 122 metabolites remained after filter by frequency and filter by sample variation (10%) respectively. PCA showed on x, y and z axis captured almost 67% of the variance in total (Fig. 6.8). According to Figure 6.8, a clear grouping of control flesh and control heart was observed. However, flesh and heart tissues with BCL discoloration were grouped together (Fig. 6.8). Statistical analysis was carried out using two-way ANOVA pairing BCL flesh with control flesh samples and BCL heart with control heart and 13 out of 54 metabolites were significant ($P < 0.05$) (Appendix D; Table 2.7). Fold change analysis results on the same pairing revealed that 12 metabolites including unsaturated hydroxy fatty acids were all down regulated in BCL samples regardless of the tissue and that happened due to no changes in all those metabolites in BCL samples (Fig. 6.9).

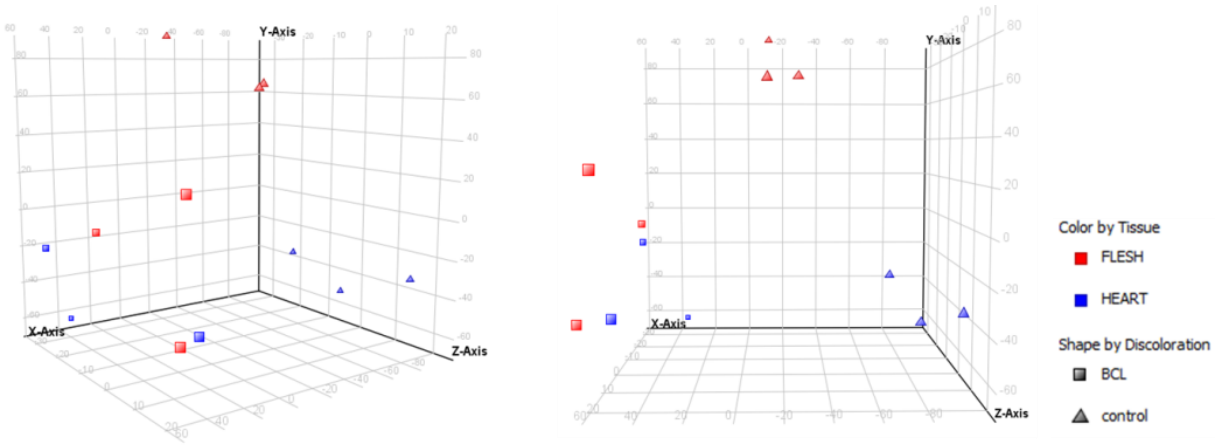


Figure 6.8 3D Principal Component Analysis scatter plot showing differences between BCL (brown centre light) and control tissue samples of stock 23 (susceptible to BH) in negative mode (x= 30.75%, y= 25.95%, z= 10.29%).

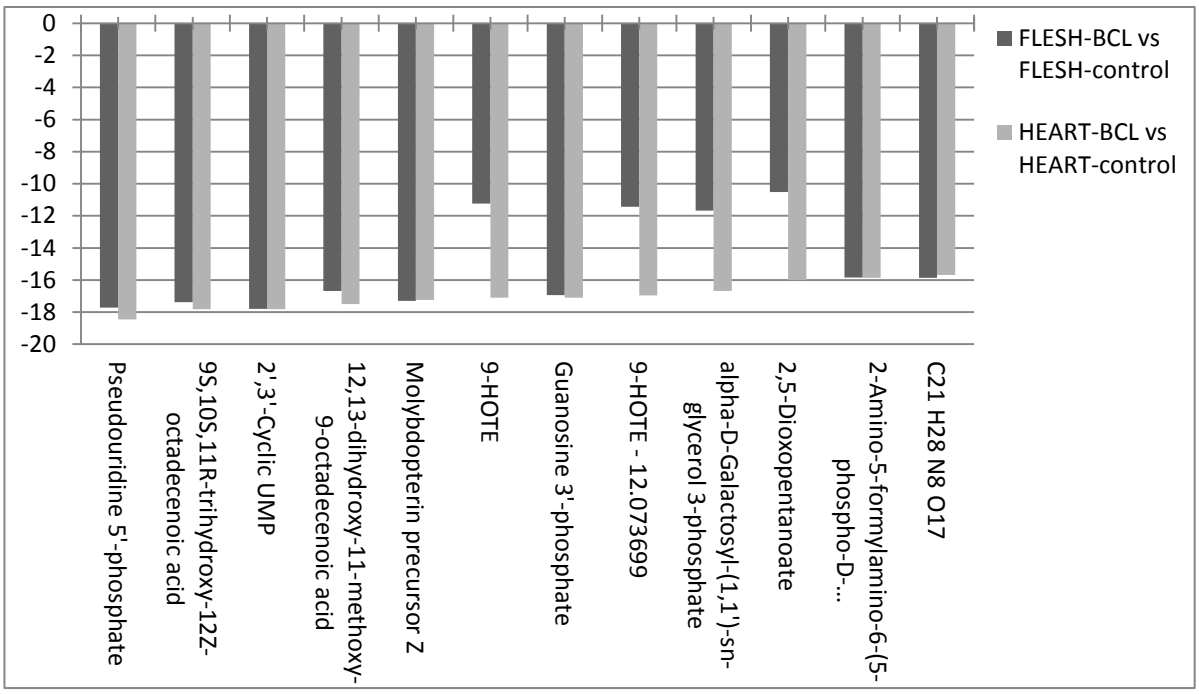


Figure 6.9 Fold change analysis results of 'tissue vs. discoloration' interpreting BCL (brown centre light) vs. control in 'experiment A'. Metabolite regulation (log FC normalized) is shown in negative mode (Appendix D; Table 2.8).

The last interpretation of the 'experiment A' was between tissue samples with pith discoloration against control samples. Filter by frequency resulted in 128 out of 374

metabolites that were additionally filtered by sample variability (10%) and 68 metabolites finally remained. PCA showed a clear separation of tissue and discoloration conditions on x, y and z axis capturing almost 70% of the total variance (Fig. 6.10). Figure 6.10 shows a clear separation of tissue discoloration more distinctively between the pith and control heart samples. Two-way ANOVA was carried out pairing pith flesh with control flesh samples and pith heart and control heart samples showing that 10 metabolites were found to be significant ($P < 0.05$) (Appendix D; Table 2.9). Fold change analysis results were shown in Figure 6.11. Two unknown metabolites were up regulated in pith samples. However, five metabolites showed down regulation in pith samples (Fig. 6.11). Actually, chlorogenic acid, 2,5-dioxopentanotate and 4-hydroxypheylacetylglutamine showed no changes in pith samples thus a down regulation was observed when compared with control.

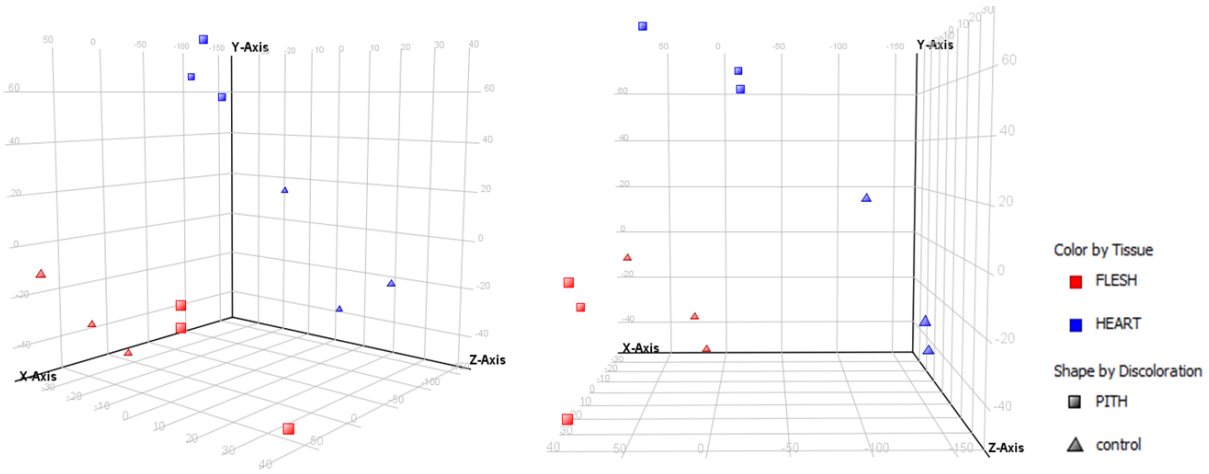


Figure 6.10 3D Principal Component Analysis scatter plot showing differences between pith and control tissue samples of stock 23 (susceptible to BH) in negative mode (x= 39.5%, y= 19.12%, z= 10.69%).

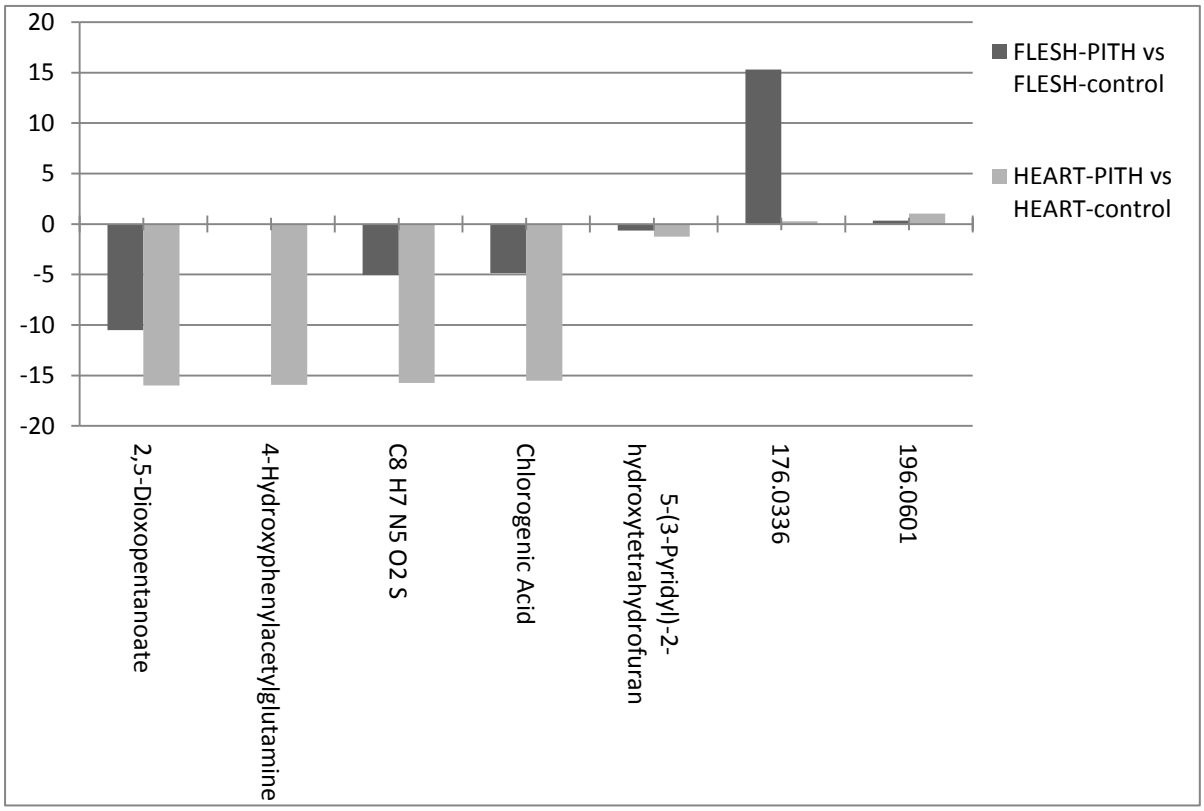


Figure 6.11 Fold change analysis results of 'tissue vs. discoloration' interpreting pith vs. control in 'experiment A'. Metabolite regulation (log FC normalized) is shown in negative mode (Appendix D; Table 2.10).

Positive ionization mode

In general, 2516 known and un-known metabolites in total were detected. Interpreting discolorations (BC, BCL, BH or pith) with control irrespective of tissue condition. Filtering by frequency reduced the number of total metabolites to 517 that were further reduced to 151 metabolites after filter by sample variability (10%). The 3D PCA on the data showed a separation of tissue discoloration on x, y and z axis (capturing ca. 53% of the variance in total). The separation of the tissue discoloration followed a similar pattern as it has also been seen in negative mode (Fig. 6.12). According to Figure 6.12, BH and BCL discolorations and control were grouped well, but a distinct group of BC and pith discoloration was not shown. Moderated T-test was used as statistical tool comparing each discoloration with the control

resulting in 42 significant metabolites ($P < 0.001$) (Appendix D; Table 3.1). Fold change analysis indicated that 33 metabolites varied in regulation between the samples analysed (Table 6.4).

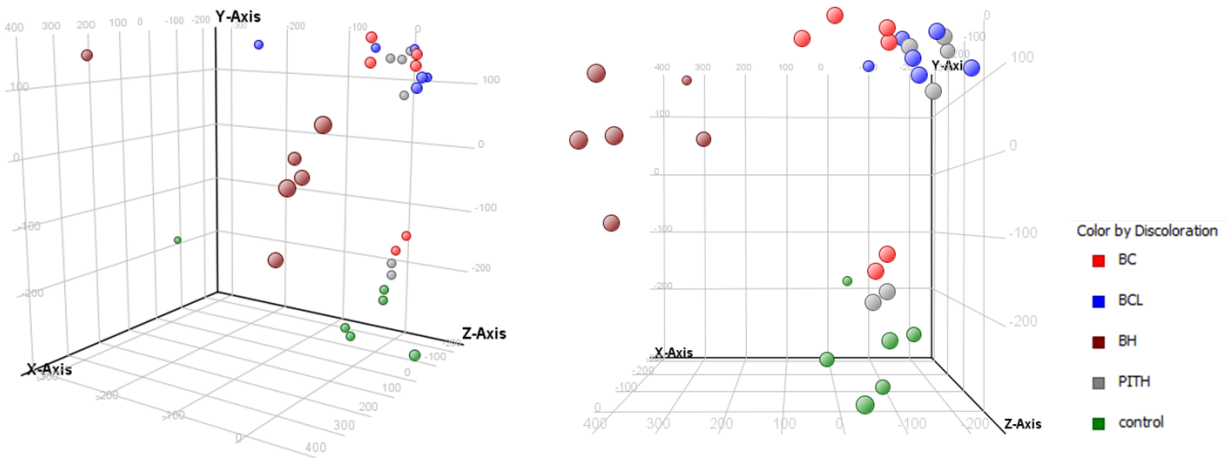


Figure 6.12 3D Principal Component Analysis scatter plot showing differences between control and tissue discolorations of stock 23 (susceptible to BH) in positive mode (x= 23.25%, y= 19.37%, z= 10.65) (BC, brown centre; BCL, brown centre light; BH, dark brown to black).

Table 6.1 Fold-change analysis results of 'discoloration only' interpretation in 'experiment B' of year 1. Metabolite regulation (log FC normalized) is shown in positive mode (BC, brown center; BCL, brown center light; BH, dark brown to black; C, control) (Appendix D; Table 3.2) .

Metabolite	BC vs C	log fc	BCL vs C	log fc	BH vs C	log fc	PITH vs C	log fc
1434.9309@6.42445	up	5.24	up	8.03	down	-7.89	up	8.10
1508.1241@4.6621003	down	-0.02	down	-5.40	up	11.47	down	-5.40
16,16-dimethyl-PGD2	down	-16.00	down	-16.00	down	-13.31	down	-16.00
1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine N-oxide	down	-15.82	down	-5.51	down	-15.82	down	-15.82
4-Nitrotoluene	down	-3.09	down	-2.88	down	-16.39	down	-0.31
4'-Prenyloxyresveratrol	down	-3.33	down	-0.35	down	-16.45	down	-0.16
6Z-Octene-2,4-diynoic acid	down	-0.06	up	0.18	down	-10.88	up	0.26
6Z-Octene-2,4-diynoic acid + 1.3391001	up	5.46	up	15.99	down	0	up	5.45
816.8409@5.68775	up	5.28	down	0	up	16.30	down	0
817.042@5.688111	up	2.76	down	-2.56	up	13.87	down	-2.56
8-Hydroxyadenine	down	-10.98	down	-16.54	down	-16.54	down	-11.17
8-methoxy-13-hydroxy-9,11-octadecadienoic acid	down	-14.26	down	-16.95	down	-16.95	down	-11.47
9-HOTE	up	2.54	up	5.12	up	17.31	up	2.55
9S,10S,11R-trihydroxy-12Z-octadecenoic acid	down	-0.26	down	-0.36	up	1.84	down	-0.08
Adenine	down	0	down	0	up	19.28	down	0
C7 H13 N	down	-0.14	up	0.04	down	-11.17	up	0.06
C8 H9 N	up	11.86	down	-5.86	up	8.47	up	11.67

C9 H6 O3	down	-0.09	up	0.18	down	-1.24	up	0.23
Chlorogenic Acid	down	-0.16	up	0.13	down	-1.35	up	0.25
Gln Phe Gln	down	-5.38	down	-0.22	down	-15.56	down	-5.14
Lagochilin	down	-14.05	down	-16.70	down	-14.07	down	-11.32
Mometasone Furoate	up	2.71	up	2.80	up	17.77	up	2.78
N-(6-aminohexanoyl)-6-aminohexanoic acid	down	-11.42	down	-17.14	down	-0.05	down	-11.46
N-(6-aminohexanoyl)-6-aminohexanoic acid + 2.6753333	down	0	down	0	up	16.72	down	0.00
N-(6-aminohexanoyl)-6-aminohexanoic acid + 3.180625	down	-11.51	down	-17.15	down	-0.74	down	-11.49
N-Hydroxypentobarbital	up	0.03	down	-2.56	up	14.22	down	-0.01
PRIMA-1	down	-2.58	up	13.07	down	-2.58	up	0.02
Succinoadenosine	down	0	up	5.07	up	17.10	down	0
Trp Asp Gly	down	-5.16	down	-5.13	up	9.48	down	-7.76
Trp Ser Gln	up	10.32	up	16.14	down	0	up	10.44
Val Ile	down	-11.13	down	-16.62	up	0.36	down	-11.22
Val Ile + 1.5134287	down	-2.63	down	-2.63	up	14.66	down	-2.63
Val Ile + 1.953625	down	-5.28	down	-5.28	up	11.84	down	-5.28

Interpreting BH against control samples in positive mode, 688 out of 2156 and 439 out of 688 metabolites remained after filtering by frequency and filter by sample variation (10%), respectively. According to PCA, almost 65% of the variance in total showed in x, y and z axis and clear groups of tissue and discoloration conditions was observed (Fig. 6.13). Two-way ANOVA was performed on 439 metabolites pairing BH flesh with control flesh samples and BH heart with control heart samples resulting in 26 significant metabolites ($P < 0.001$) (Appendix D; Table 3.3). Fold change analysis results showed that those 26 metabolites varied in regulation (Fig. 6.14 and 6.15). According to Figure 6.14, three fatty acids were down regulated in BH flesh samples and five metabolites including Alpha-CEHC (or 2,5,7,8-tetramethyl-2-(2'-carboxyethyl)-6-hydroxychroman; major metabolite of a-Tocopherol) were all up regulated in flesh BH samples. Similar metabolite regulations were observed in heart BH samples where fatty acids were down regulated and Alpha-CEHC and 4-oxo-nonenal (lipid peroxidation product) were up regulated (Fig. 6.15).

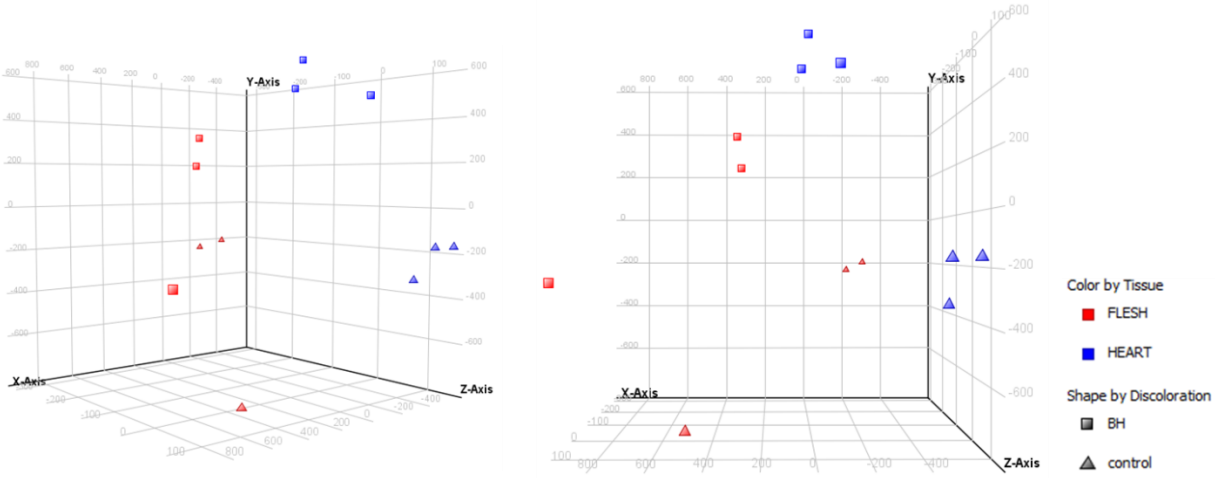


Figure 6.13 3D Principal Component Analysis scatter plot showing differences between BH (dark brown to black) and control tissue samples of stock 23 (susceptible to BH) in positive mode (x= 28.71%. y= 26.08%, z= 10.04%).

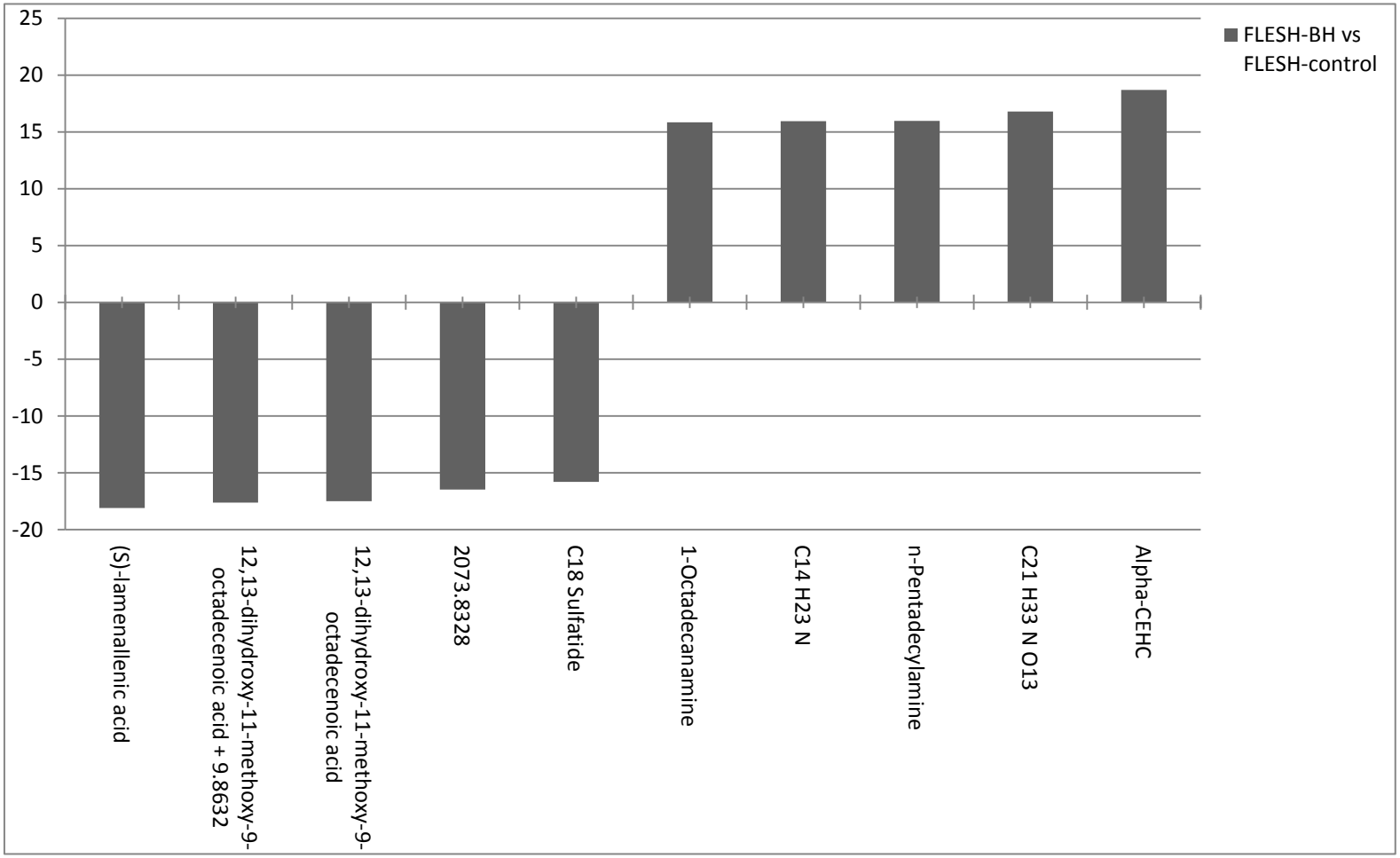


Figure 6.14 Fold change analysis results of 'tissue vs. discoloration' interpreting flesh tissue with BH (dark brown to black) vs. control flesh tissue in 'experiment B'. Metabolite regulation (log FC normalized) in is shown positive mode (Appendix D; Table 3.4).

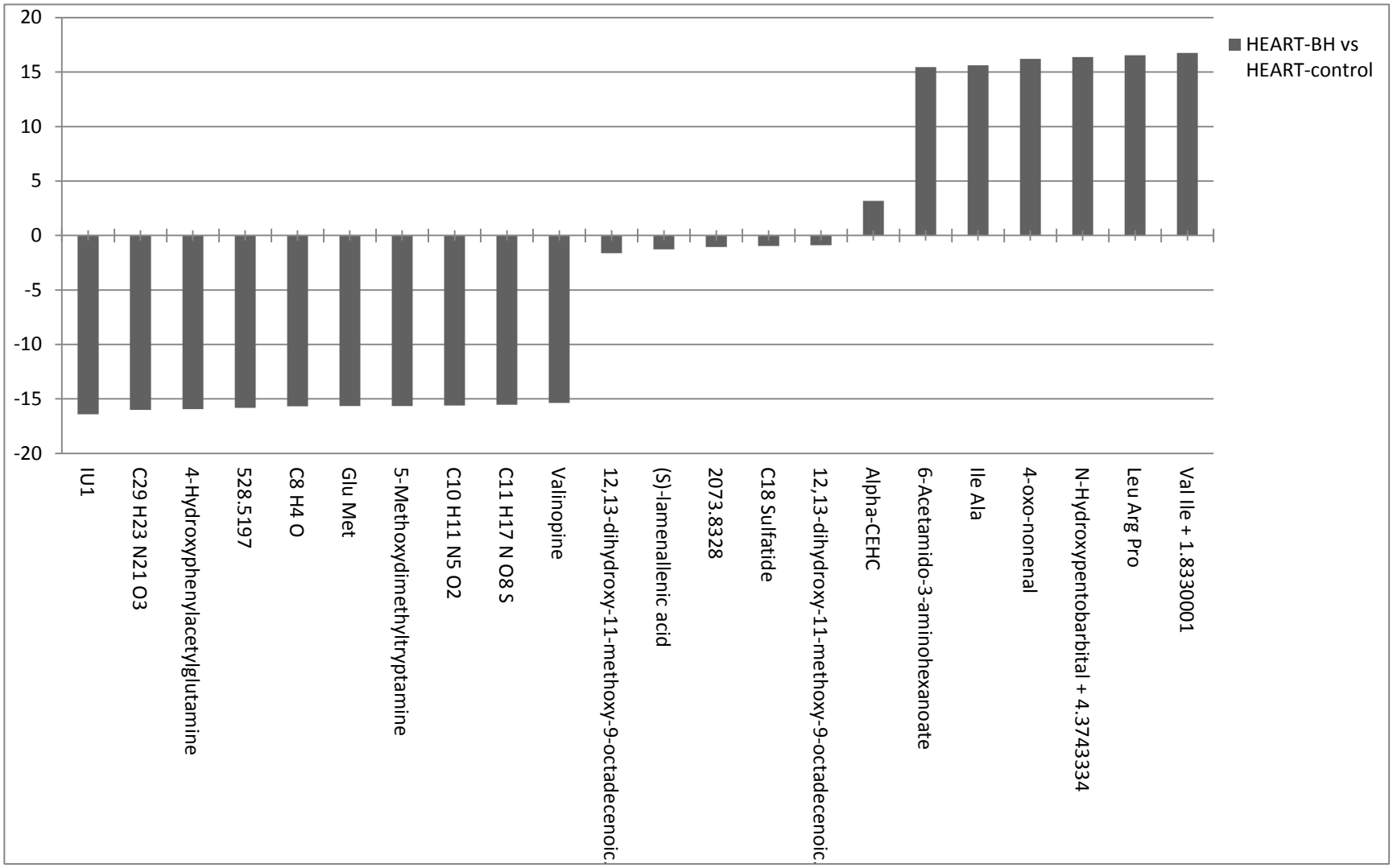


Figure 6.15 Fold change analysis results of 'tissue vs. discoloration' interpreting BH (dark brown to black) vs. control (heart tissue) in 'experiment B'. Metabolite regulation (log FC normalized) is shown in positive mode (Appendix D; Table 3.4).

The following interpretation was between tissue samples with BC against control tissue samples. Filter by frequency resulted in 626 out of 2156 entities where they were additionally filtered by sample variability (10%) and 357 entities remained. PCA showed a clear separation of tissue and discoloration conditions on x, y and z axis capturing almost 64% of the variance in total. Figure 6.16 shows a clear separation between flesh and heart tissues with BC discoloration. Control samples showed a clear clustering as well (Fig. 6.16). Two-way ANOVA was carried out pairing BC flesh with control flesh samples and BC heart and control heart samples and 7 significant metabolites were indicated with a probability of $P < 0.001$ (Appendix D; Table 3.5). Those 7 metabolites including a-CEHC were varied in regulation after fold change analysis (Fig. 6.17).

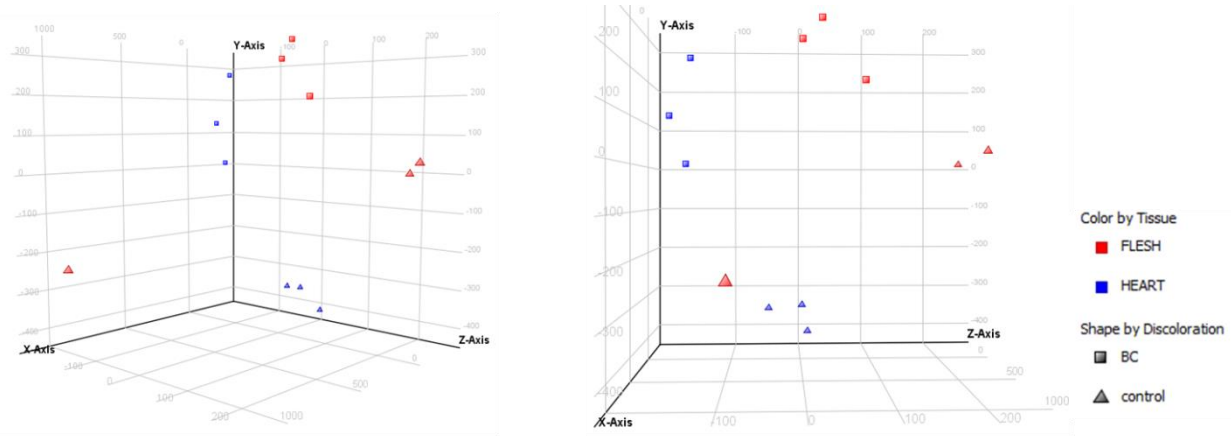


Figure 6.16 3D Principal Component Analysis scatter plot showing differences between BC (brown centre) and control tissue samples of stock 23 (susceptible to BH) in positive mode (x= 30.95%, y= 21.12%, z= 11.71%).

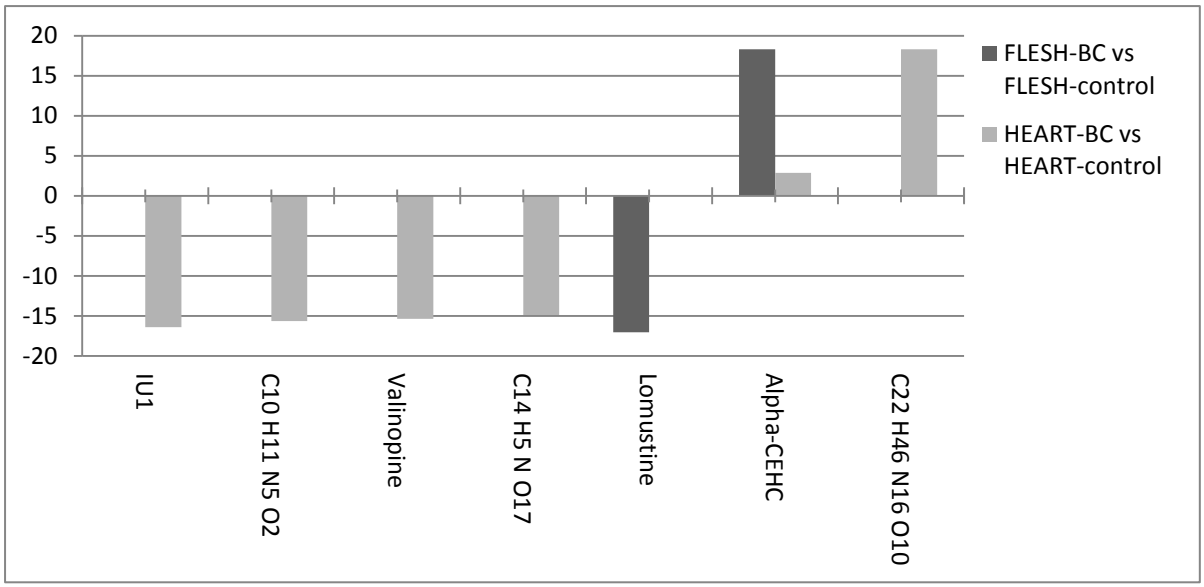


Figure 6.17 Fold change analysis results of 'tissue vs. discoloration' interpreting BC (brown center) vs. control in 'experiment B'. Metabolite regulation (log FC normalized) is shown in positive mode (Appendix D; Table 3.6).

Interpreting tissue samples with BCL against control tissue samples, filtering by frequency reduced the number of total metabolites (2156) to 628 that were further reduced to 386 metabolites after filter by sample variability (10%). According to Figure 6.18, 63% of the total variance was observed on x, y and z axis showing a separation between BCL discoloration and control. Control heart and flesh tissue samples were well separated; however BCL flesh and heart tissue samples were grouped closely (Fig. 6.18). Two-way ANOVA was performed pairing BCL flesh with control flesh samples and BCL heart with control heart samples resulting in 12 significant metabolites ($P < 0.01$) (Appendix D; Table 3.7). Fold change analysis results were shown in Figure 6.19.

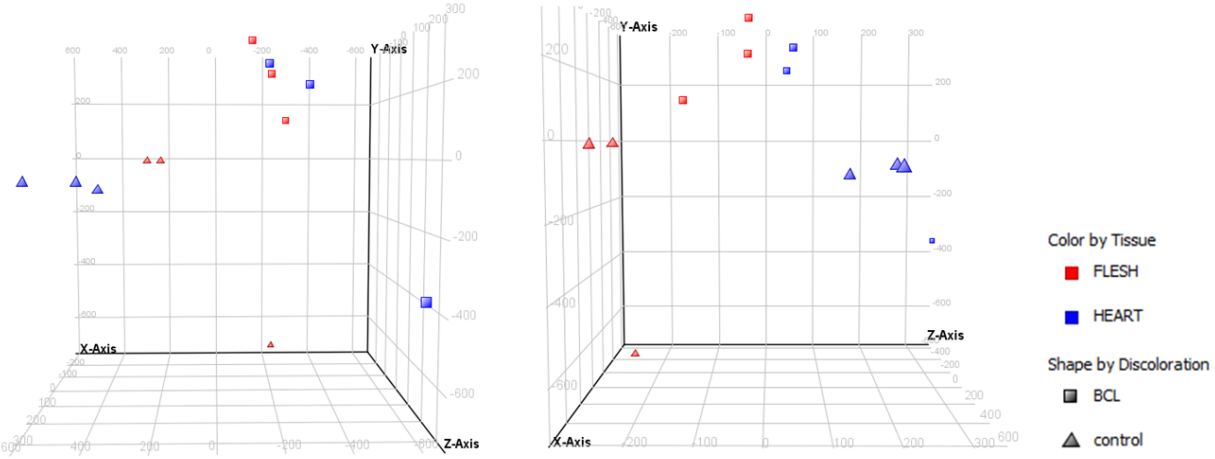


Figure 6.18 3D Principal Component Analysis scatter plot showing differences between BCL (brown centre light) and control tissue samples of stock 23 (susceptible to BH) in positive mode (x= 27.05%, y= 21.33%, z= 14.33%).

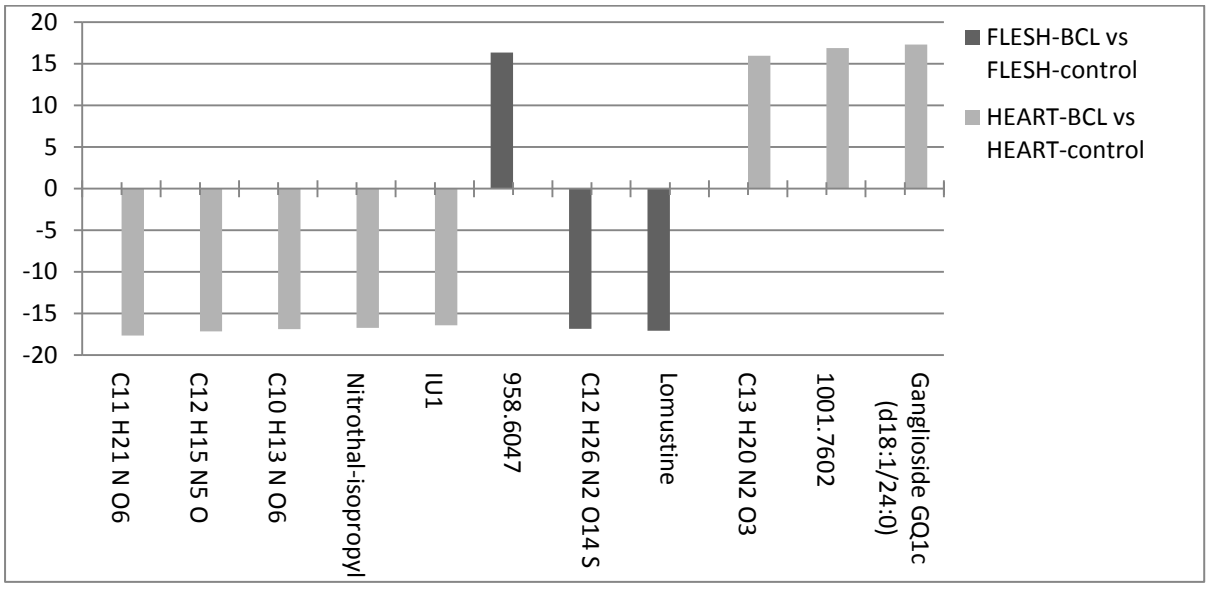


Figure 6.19 Fold change analysis results of 'tissue vs. discoloration' interpreting BCL (brown center light) vs. control in 'experiment B'. Metabolite regulation (log FC normalized) is shown in positive mode (Appendix D; Table 3.8).

In positive mode, quality control on samples with pith discoloration against control samples indicated 622 out of 2156 and 349 out of 622 metabolites after filter by frequency and filter by sample variation (10%) respectively. The PCA on the data showed a separation

of samples on x, y and z axis capturing, in total, almost 65% of the variance (Figure 6.20). A clear clustering of control heart samples was observed. However flesh and heart tissue samples with pith discoloration and control flesh were clustered closely (Fig. 6.20). After performing two-way ANOVA pairing pith flesh with control flesh and pith heart with control heart samples only 12 significant metabolites with a significance of $P < 0.01$ remained (Appendix D; Table 3.9). Fold change analysis results showed that all 12 metabolites varied in regulation mainly in pith heart samples (Fig. 6.21).

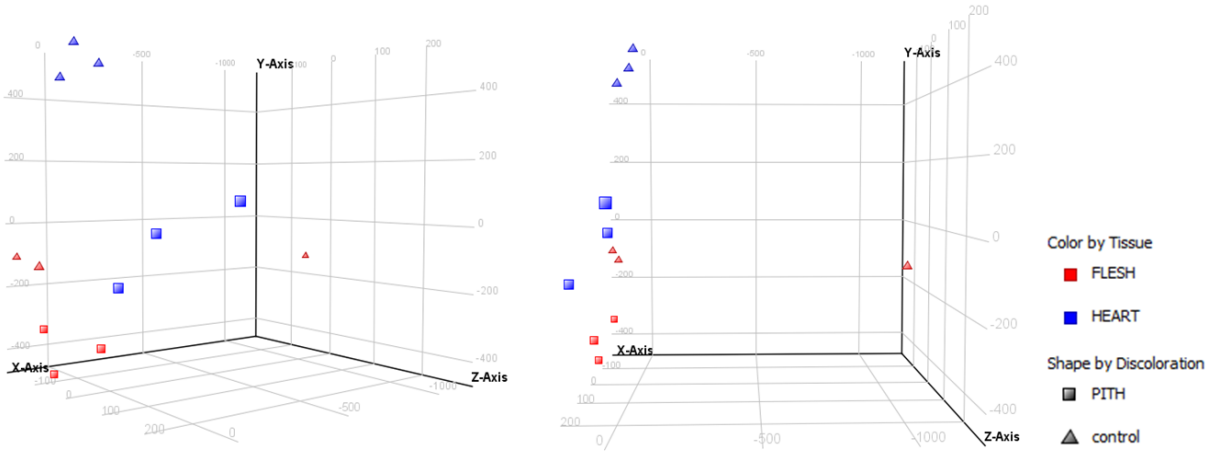


Figure 6.20 3D Principal Component Analysis scatter plot showing differences between pith and control tissue samples of stock 23 (susceptible to BH) in positive mode (x= 28.96%, y= 25.79%, z= 10.76%).

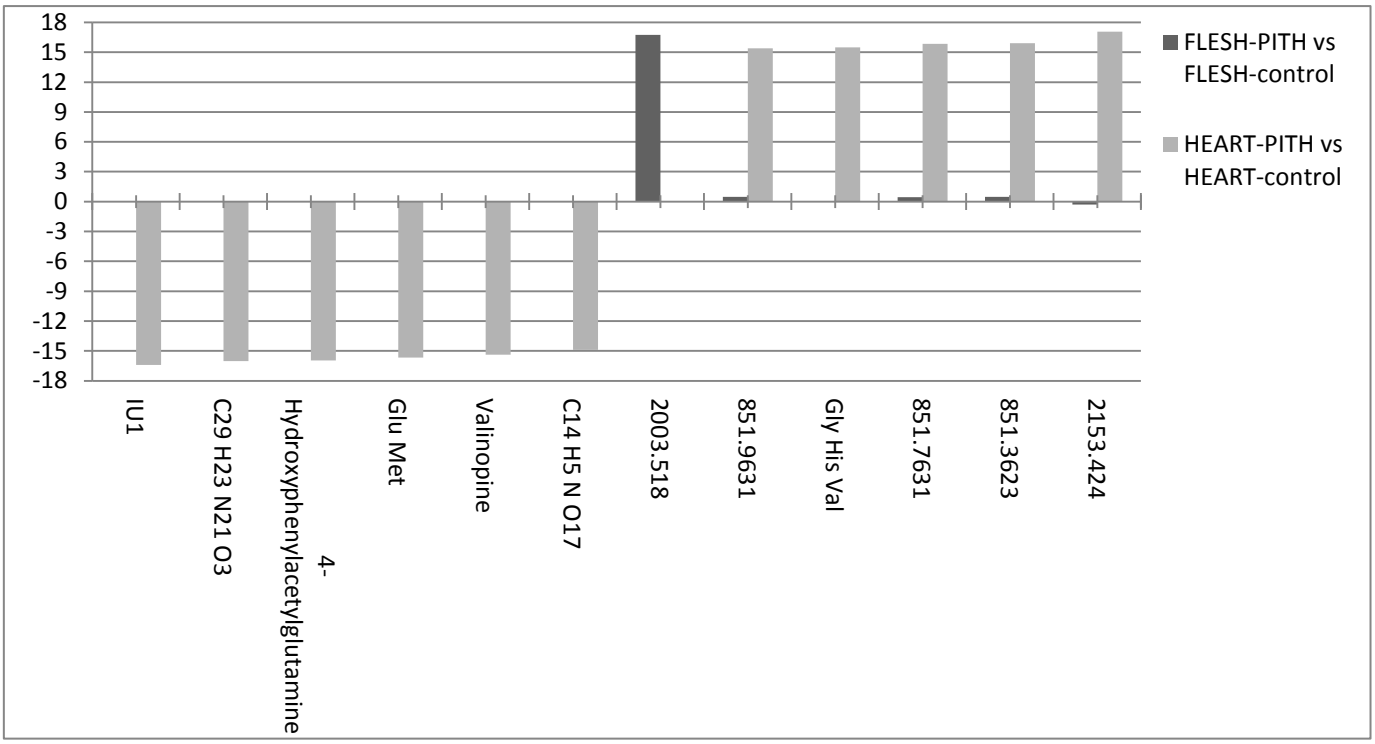


Figure 6.21 Fold change analysis results of 'tissue vs. discoloration' interpreting pith vs. control in 'experiment B'. Metabolite regulation (log FC normalized) is shown in positive mode (Appendix D; Table 3.10).

6.3.2. Metabolomic differences between control samples of potato stocks with different susceptibility to blackheart disorder

In both 'experiments C and D' (in negative and positive mode respectively), 18 control samples (n = 8 flesh and 10 heart) of stock 23 (susceptible to BH) against 23 (n = 11 flesh and 12 heart) control samples of stock 12 (non-susceptible to BH) were used for analysis. 'Susceptibility only' interpretation was carried out regardless the tissue condition (18 against 23 samples), while 'tissue vs. susceptibility' interpretation was conducted comparing flesh samples of stock 23 (n = 8) against flesh samples of stock 12 (n = 11) and heart samples of stock 23 (n = 10) against heart samples of stock 12 (n = 12).

Negative ionization mode

Generally, 832 known and un-known metabolites were identified in this 'experiment B' in negative ion mode. Regarding the 'susceptibility only' interpretation (irrespectively the tissue) metabolites were filtered by frequency and 103 reproducible entities remained after filtering by sample variability (25%). PCA followed where all the possible principal components were calculated and visually represented per susceptibility condition coloured-coded in a 3D scatter plot (Fig. 6.22). The PCA on the data showed a clear separation of potato stock susceptibility to BH on x, y and z axis (capturing it total 71% of the variance) (Fig. 6.22). Statistical analysis was performed using Moderated t-test comparing control samples of stock 23 (susceptible to BH) against control samples of stock 12 (non-susceptible to BH) with a probability of $P < 0.05$ resulting in 14 known and unknown significant metabolites (Appendix D; Table 4.1). Fold change analysis showed that 8 out of 14 metabolites were significantly up or down regulated. Quinic acid was down regulated, but two polyhydroxyflavones [(3,5,7,8-tetrahydroxy-2-(3,4,5-trihydroxy phenyl)chromen-4-one or hibiscetin and 5,7,3',4',5'-Pentahydroxy-3,6,8-trimethoxyflavone] were both up-regulated in the stock with BH susceptibility (Fig. 6.23).

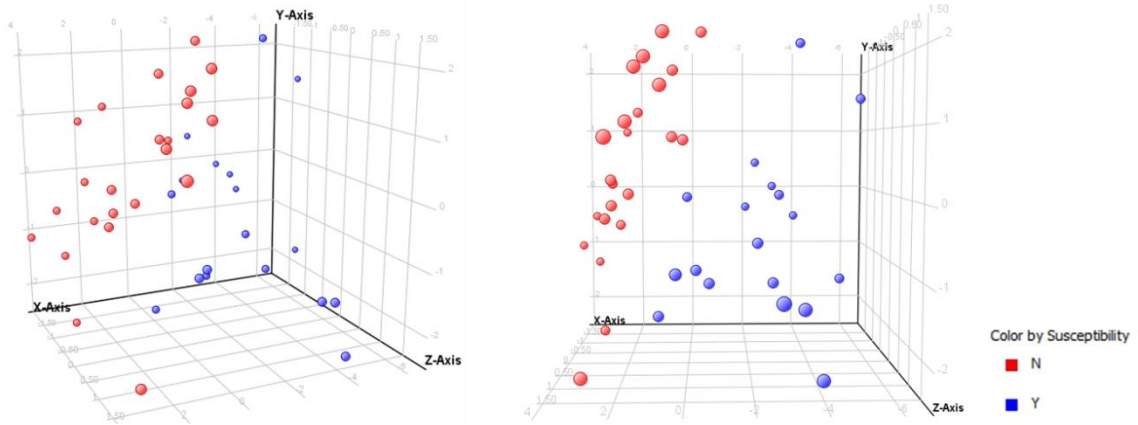


Figure 6.22 3D Principal Component Analysis scatter plot showing differences between potato stock susceptibility in negative mode [N = stock 12 (non-susceptible to BH), Y = stock 23 (susceptible to BH)] (x= 50.7%, y= 11.71%, z= 8.6%).

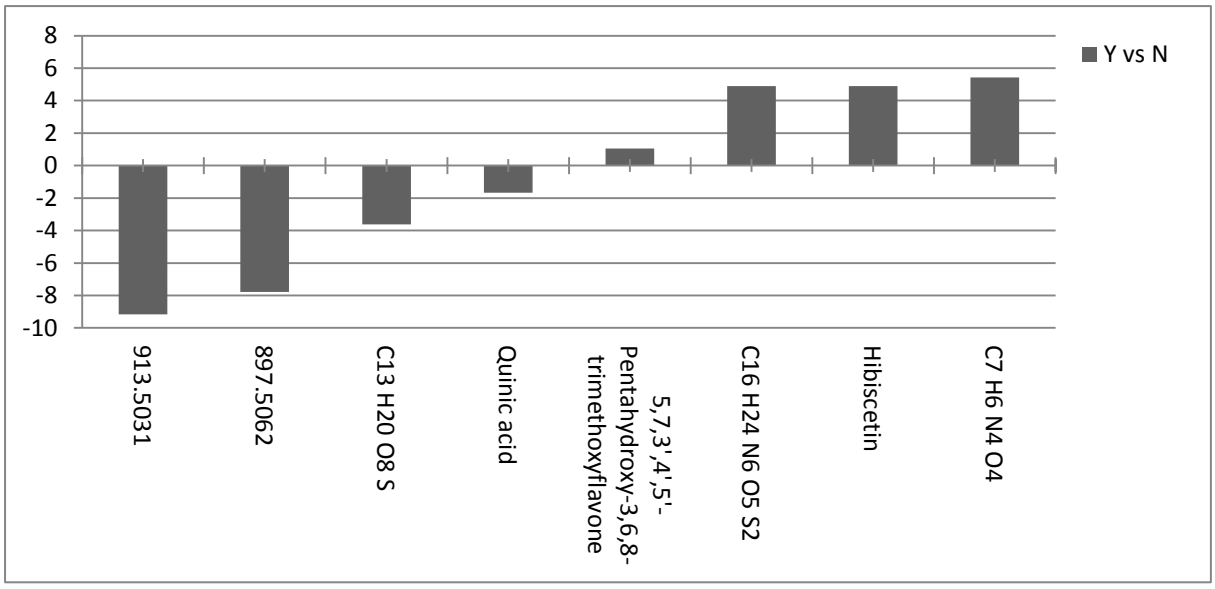


Figure 6.23 Fold change analysis results of 'susceptibility only' interpretation in 'experiment C'. Metabolite regulation (logFC normalized) is shown in negative mode (Y = susceptible stock 23, N = non-susceptible stock 12) (Appendix D; Table 4.2).

Regarding the interpretation between tissue vs. susceptibility conditions, filtering by frequency decreased the number of total metabolites (n = 832) to 157 that were further reduced to 30 metabolites after filter by sample variability (10%). According to Figure 6.24, PCA on data showed a good separation of stock susceptibility on x axis (capturing ca. 29% of the variance) and y and z axis only captured a further 14 and 9% of the variance. There was a good grouping of flesh and heart samples of stock 12. However, clear groups of flesh and heart samples of stock 23 were less distinguished (Fig. 6.24). Two-way ANOVA was performed on samples pairing flesh tissue samples of stock 23 against flesh tissue samples of stock 12 and heart tissue samples of stock 23 against heart tissue samples of stock 12. Results showed one significant unknown metabolite [m/z= 887.4782 and retention time= 5.8079376] ($P < 0.01$) that was up regulated in flesh and heart samples of stock 12 (non-susceptible to BH) (Appendix D; Table 4.3 and 4.4).

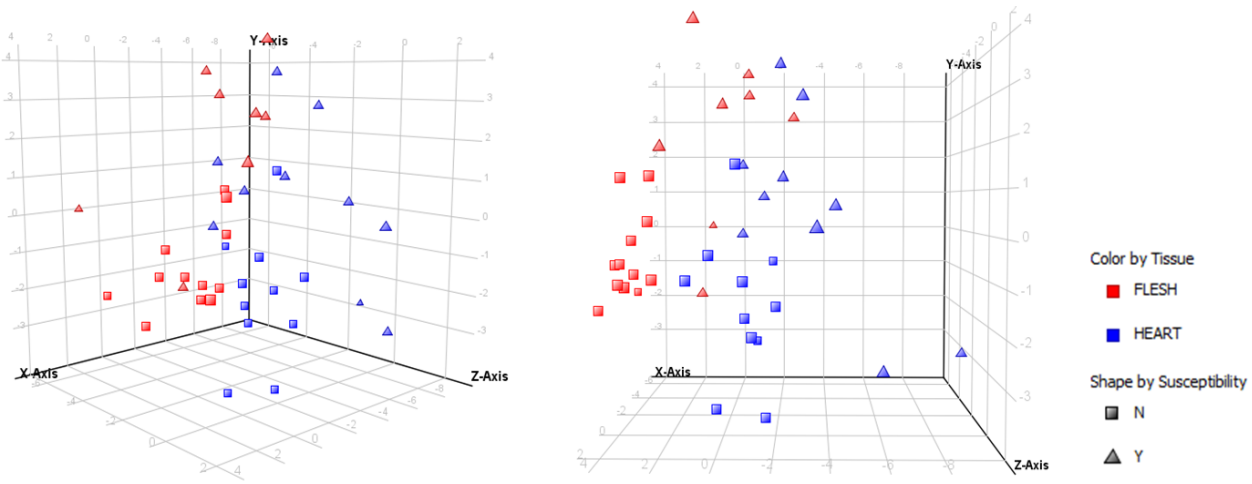


Figure 6.24 3D Principal Component Analysis scatter plot showing differences between tissues of potato stocks with different susceptibility to BH in negative mode [N = stock 12 (non-susceptible to BH), Y = stock 23 (susceptible to BH)] (x= 28.78%, y= 13.81%, z= 8.97%).

Positive ionization mode

In general, 845 known and un-known metabolites were identified in this 'experiment D' in positive ion mode. In terms of the 'susceptibility only' interpretation (irrespectively the tissue), metabolites were filtered by frequency and 103 reproducible entities remained with a sample variability of 25%. PCA followed where all the possible principal components were calculated and visually represented per susceptibility condition colour-coded in a 3D scatter plot (Fig. 6.25). According to PCA, 83% of variance in total was explained on x, y and z axis and a partial separation according to stock susceptibility to BH on x axis (captured 53% of variance) was observed (Fig. 6.25). Moderated t-test comparing control samples of stock 23 (susceptible to BH) against control samples of stock 12 (non-susceptible to BH) was performed resulting in 10 significant metabolites with a probability of $P < 0.05$ (Appendix D; Table 5.1). Fold change analysis showed that 7 out of 10 metabolites have been significantly regulated. Solanine and alpha-chaconine (glycoalkaloids) and 4-oxoproline were down-regulated in stock 23, but phenylpropiolic acid was up regulated (Fig. 6.26).

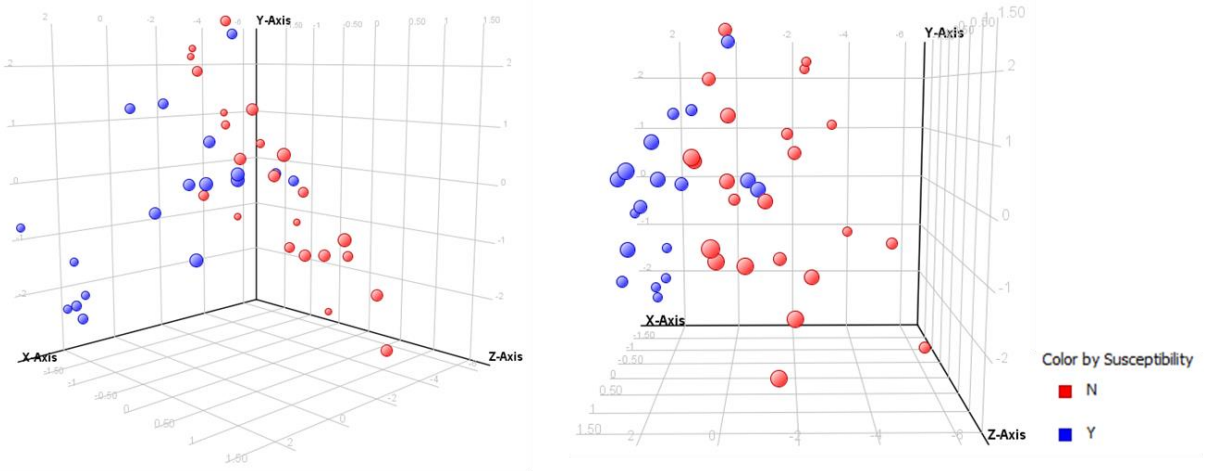


Figure 6.25 3D Principal Component Analysis scatter plot showing differences between potato stock susceptibility to in positive mode [N= stock 12 (non-susceptible to BH), Y= stock 23 (susceptible to BH)] (x= 53.4%, y= 19.23%, z= 10.35%).

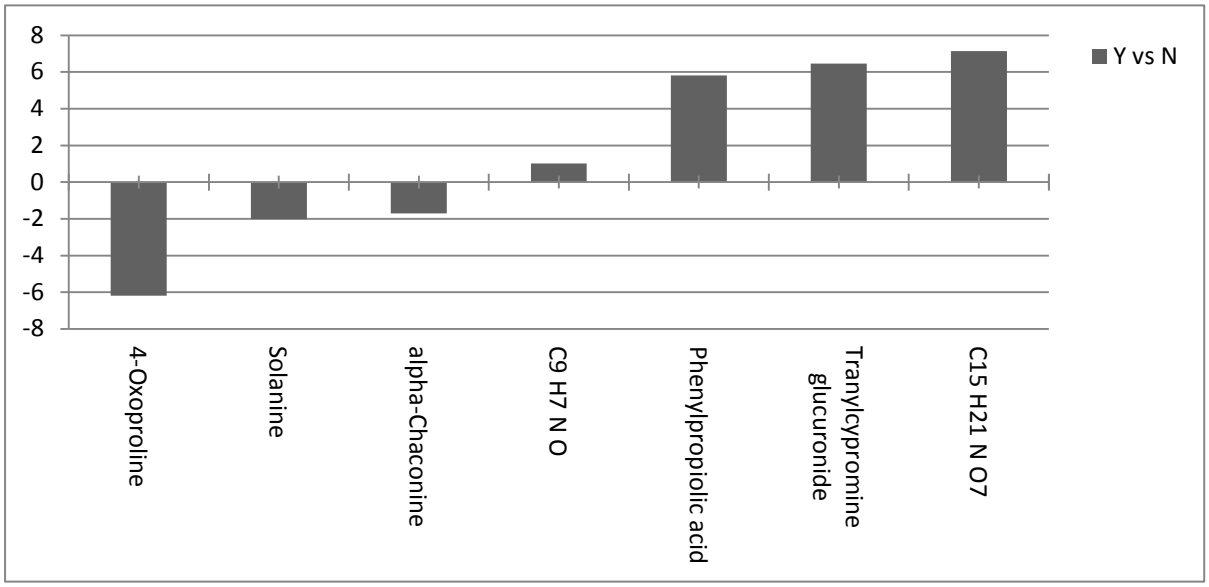


Figure 6.26 Fold change analysis results of 'susceptibility only' interpretation in 'experiment D'. Metabolite regulation (log FC normalized) is shown in positive mode (Y = susceptible stock 23, N = non-susceptible stock 12) (Appendix D; Table 5.2).

In terms of the interpretation between tissue and susceptibility conditions, filtering by frequency reduced the number of total metabolites (n = 845) to 195 that were further reduced to 20 metabolites after filter by sample variability (10%). PCA on data showed a clear separation of tissue on x axis (captured ca. 62.14% of the variance) and y and z axis only

captured a further 17.88 and 4.6% of the variance (capturing 84.62% of the variance in total) (Fig. 6.27). There was a clear grouping of flesh tissue samples of stock 12. However, grouping of heart tissue samples of stock 12 were less distinguished. No clear separation between flesh and heart tissue samples of stock 23 was observed (Fig. 6.27). Two-way ANOVA was performed pairing flesh tissue samples of stock 23 against flesh tissue samples of stock 12 and heart tissue samples of stock 23 against heart tissue samples of stock 12. Results indicated 11 significant metabolites ($P < 0.01$) (Appendix D; Table 5.3) that were also up or down regulated after fold change analysis on the same sample pairing. According to Figure 6.28, all glycoalkaloids identified (solasonine, solanine and solanidine) were significantly up-regulated in flesh (mainly) and heart tissues of stock 12. In terms of phenylalanine, relatively speaking there were no changes in flesh samples of stock 12 thus a down regulation was showed when compared with flesh samples of stock 23. Moreover phenylalanine was abundant in all heart samples of stock 23 (Fig. 6.28).

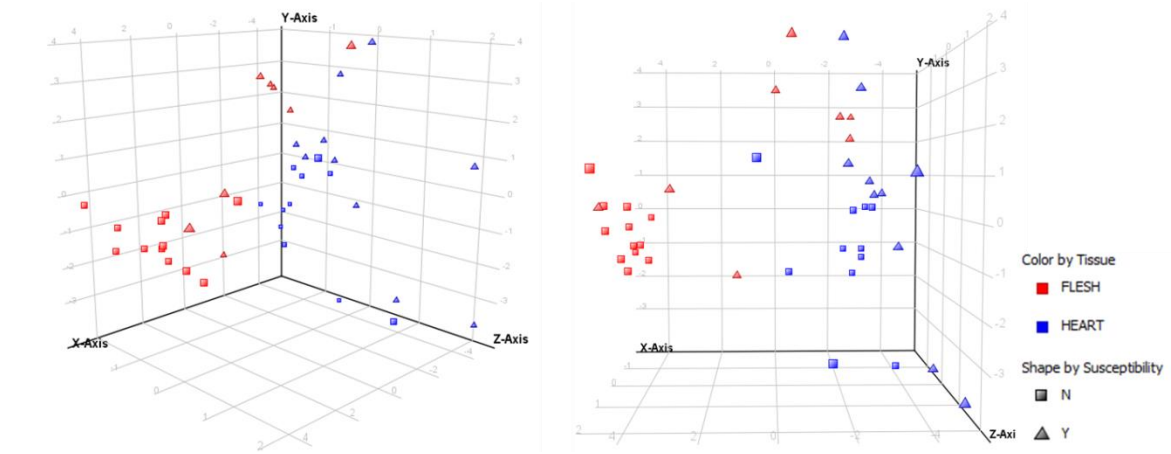


Figure 6.27 3D Principal Component Analysis scatter plot showing differences between tissues of potato stocks with different susceptibility in positive mode [N = stock 12 (non-susceptible to BH), Y = stock 23 (susceptible to BH)] (x= 62.14%, y= 17.68%, z= 4.6%).

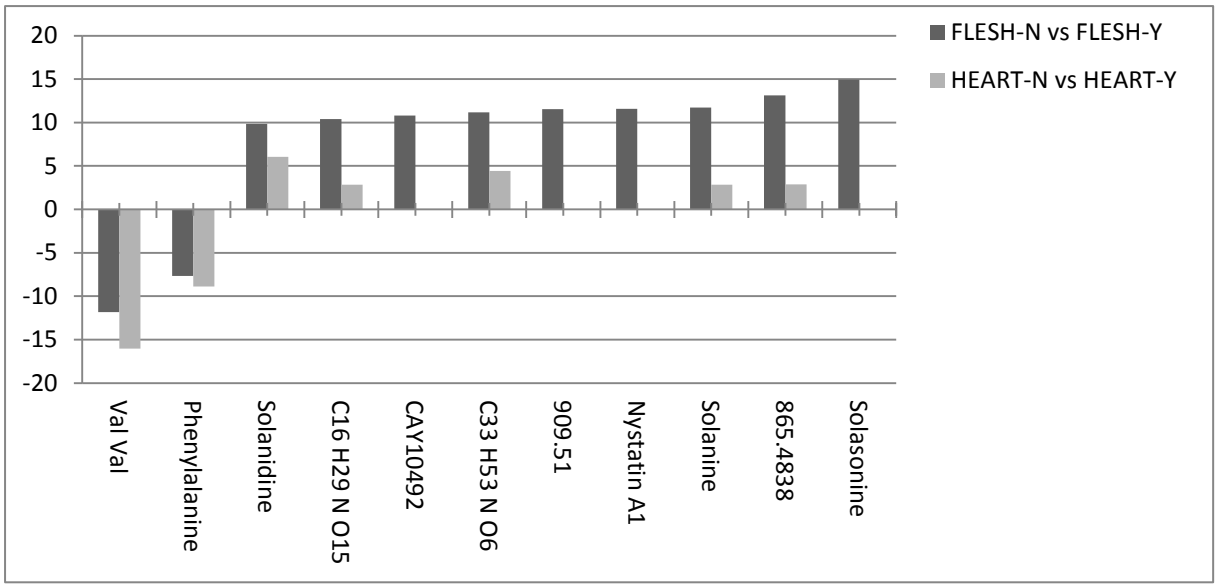


Figure 6.28 Fold change analysis results of 'tissue vs. susceptibility' interpretation in 'experiment D'. Metabolite regulation (log FC normalized) is shown in positive mode (Y = susceptible stock 23, N = non-susceptible stock 12 (Appendix D; Table 5.4).

B) Year 2 (2012-2013)

6.3.3 Metabolomic differences between affected and non-affected potato tuber tissues

In both 'experiments E and F' (in negative and positive mode respectively), 32 samples with tissue discoloration (affected) (n = 16 flesh and 16 heart) and 31 control samples (n = 15 flesh and 16 heart) of stock 7 (susceptible to BH) were used for analysis. 'Discoloration only' interpretation was carried out regardless the tissue condition (32 affected against 31 control samples), while 'tissue vs. discoloration' interpretation was conducted comparing affected flesh samples (n = 16) against control flesh samples (n = 15) and affected heart samples (n = 16) against control heart samples (n = 16).

Negative ionization mode

In general, 1286 known and un-known metabolites in total were detected in negative mode. Firstly, in terms of the interpretation 'discoloration only' regardless tissue condition (affected against control samples of stock 7), quality control on samples showed only 67 out of 1286 metabolites remained after filtering by frequency with sample variability of less than 25%. However, those 67 metabolites were not statistically different (Appendix E; Table 2.1).

Secondly, interpreting 'tissue vs. discoloration' conditions filtering by frequency reduced the number of total metabolites to 124 based on a sample variation of less than 25%. According to PCA, almost 42% of the variance in total showed on x, y and z axis. A clear separation of tuber tissue was observed on x axis capturing ca. 23% of the variance. However, a clear separation between affected and control samples was not distinguished (Fig. 6.29). Two-way ANOVA was not performed.

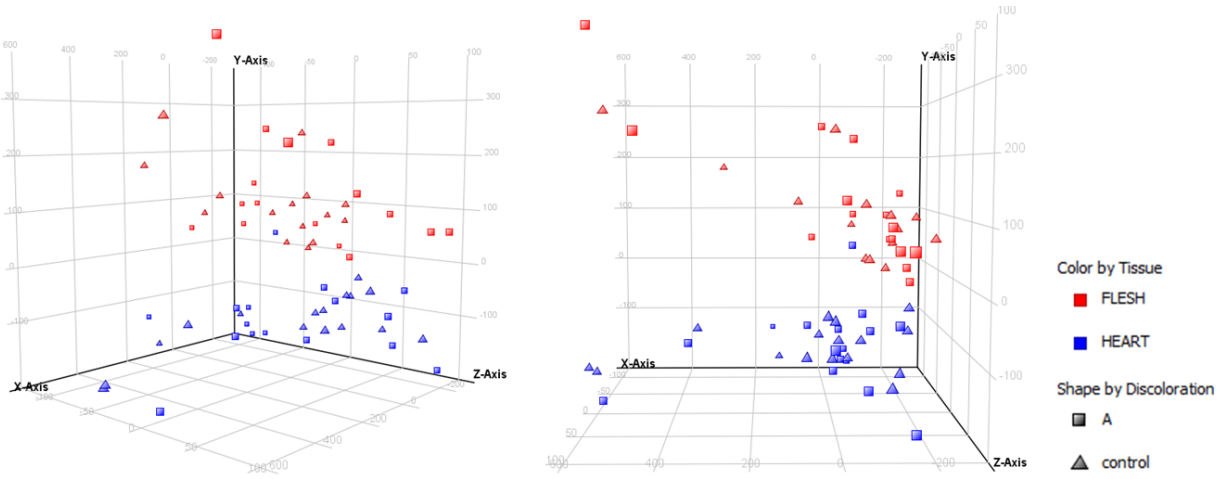


Figure 6.29 3D Principal Component Analysis scatter plot showing differences between affected and control tissue samples of stock 7 (susceptible to BH) in negative mode (A= affected, control = no discoloration) (x= 22.66%, y= 13.34%, z= 5.98%).

Positive ionization mode

Generally, 2071 known and un-known metabolites have been identified in positive mode. Interpreting the 'discoloration' condition (regardless tissue condition), quality control showed that those 2071 metabolites were reduced to 268 using filter by frequency with a coefficient of variation of less than 25% (data not shown). Nevertheless, Moderated T-test showed no significant differences.

Regarding the interpretation between 'tissue vs. discoloration' conditions, frequency filtering resulted in 370 out of 2071 metabolites that they were additionally filtered by sample variability (10%) and finally reduced to 47. PCA on data showed a clear separation between tuber tissue only, capturing almost 66% of the variance in total (Fig. 6.30). Two-way ANOVA was not performed.

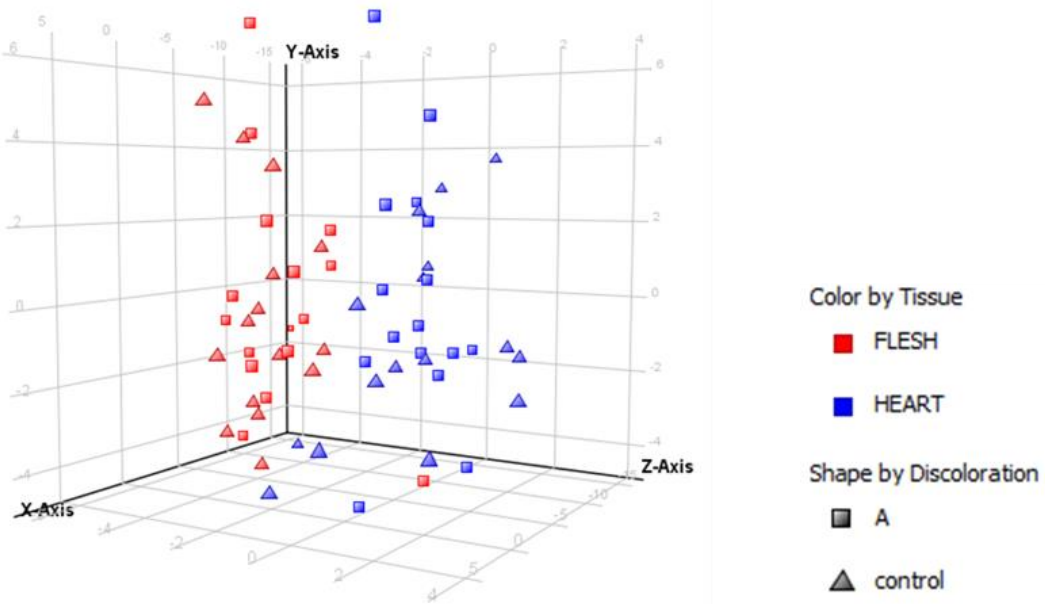


Figure 6.30 3D Principal Component Analysis scatter plot showing differences between affected and control tissue samples of stock 7 (susceptible to BH) in positive mode (A = affected, control = no discoloration) (x= 45.07%, y= 13.12%, z= 7.65%).

6.3.4. Metabolomic differences between control samples of potato stocks with different susceptibility to blackheart disorder

In both 'experiments G and H' (in negative and positive mode respectively), those 31 control samples (n = 15 flesh and 16 heart) of stock 7 (susceptible to BH) that have previously been used in those 'experiments E and F' were further compared with 32 (n = 16 flesh and 16 heart) control samples of stock 3 (non-susceptible to BH). 'Susceptibility only' interpretation was carried out regardless the tissue condition (31 against 32 samples), while 'tissue vs. susceptibility' interpretation was conducted comparing flesh samples of stock 7 (n = 15) against flesh samples of stock 3 (n = 16) and heart samples of stock 7 (n = 16) against heart samples of stock 3 (n = 16).

Negative ionization mode

Generally, 1276 known and unknown metabolites have been identified in negative mode. Interpreting 'susceptibility only' condition frequency filtering reduced the total number of metabolites (n = 1276) to 90 based on a coefficient of variation of less than 25%. PCA showed a clear separation of stock susceptibility on x, y and z axis capturing almost 45% of the total variance (Fig. 6.31). Moderated t-test was used comparing control samples of stock 7 (susceptible to BH) against control samples of stock 3 (non-susceptible to BH) resulting in 11 significant metabolites with a probability of $P < 0.001$ (Appendix E; Table 3.1). Fold change analysis with the same sample pairing indicated that those 11 metabolites were all significantly regulated. Three flavonoid glycosides and diglycosides (Myricetin 3-rutinoside, quercetin 3-glucoside-7-rutinoside and quercetin 3-glucoside-7-rhamnoside) and isoferulic acid have all been down regulated in stock 7 (susceptible to BH). The only up-

regulated metabolite in the susceptible stock was the fatty acid 9S,10S,11R-trihydroxy-12Z-octadecenoic acid (Fig. 6.32).

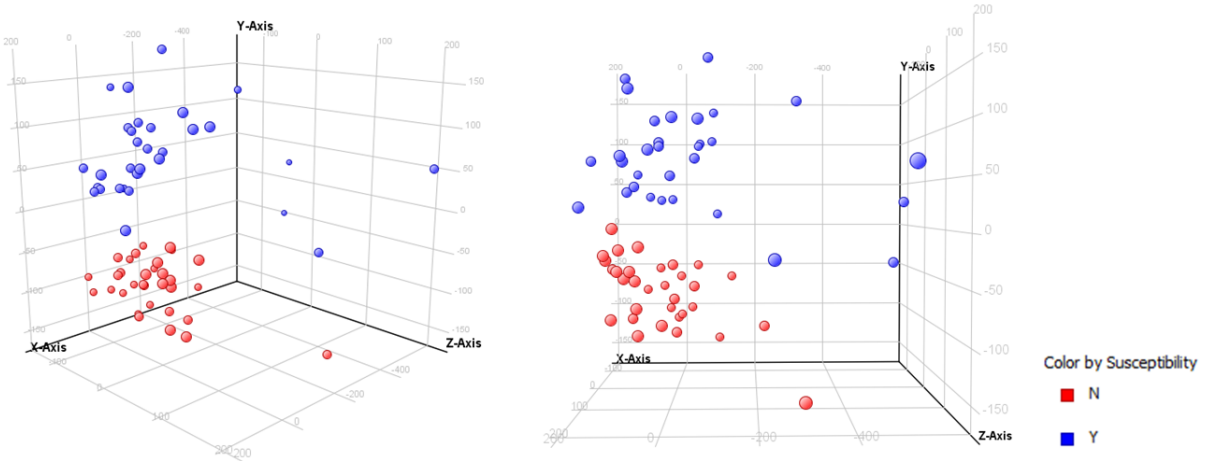


Figure 6.31 3D Principal Component Analysis scatter plot showing differences between potato stock susceptibility in negative mode [N = stock 3 (non-susceptible to BH), Y = stock 7 (susceptible to BH)] (x= 23.09%, y= 11.85%, z= 10.37%).

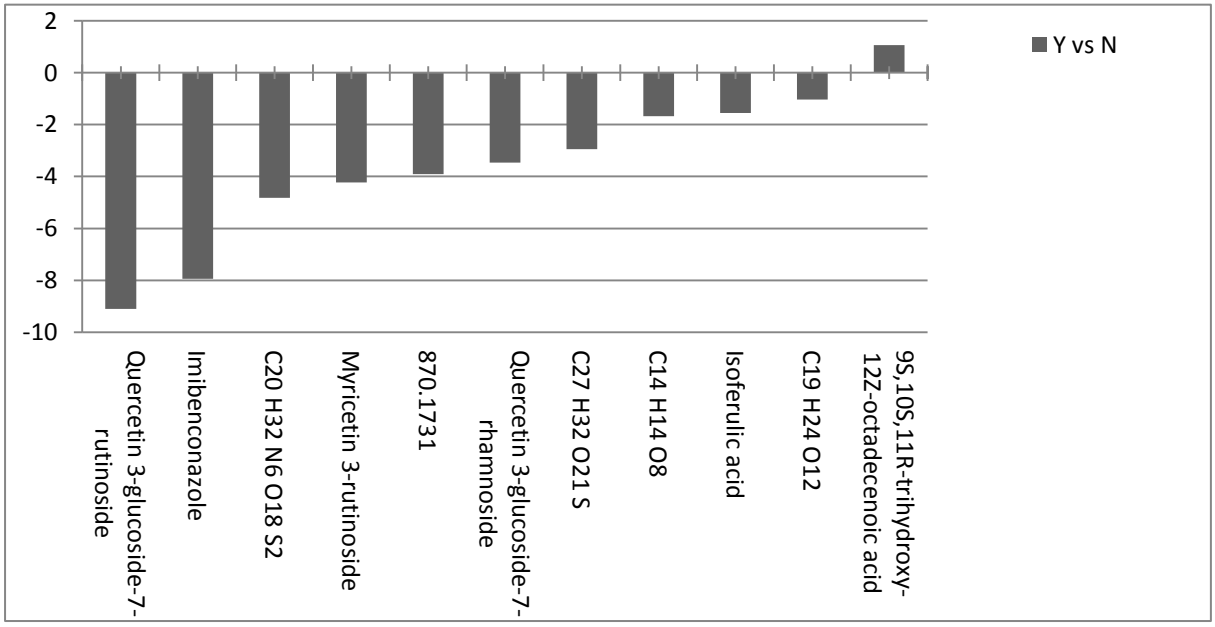


Figure 6.32 Fold change analysis results of 'susceptibility only' interpretation in 'experiment G'. Metabolite regulation (log FC normalized) is shown in negative mode (Y = susceptible stock 7, N = non-susceptible stock 3 (Appendix D; Table 5.4).

Interpretation of 'tissue vs. susceptibility' results revealed that filtering by frequency reduced the number of total metabolites (n = 1276) to 151 with sample variability of 25%. PCA on data showed a clear separation of tuber tissue and tissue discoloration on x, y and z axis capturing 23.81% of the variance in total (Fig. 6.33). Two-way ANOVA was performed pairing flesh tissue samples of stock 7 (susceptible to BH) with flesh samples of stock 3 (non-susceptible to BH) and heart tissue samples of stock 7 with stock 3 and results revealed 23 significant metabolites with a probability of $P < 0.001$. Fold change analysis results showed 16 known and unknown metabolites (Fig. 6.34). Three flavonoid glycosides and isoferulic acid were all up regulated in flesh and heart samples of stock 3 (non-susceptible to BH) thus a down regulation of those metabolites was previously observed in stock 7 (susceptible to BH) when interpreting 'susceptibility only' (Fig 6.32).

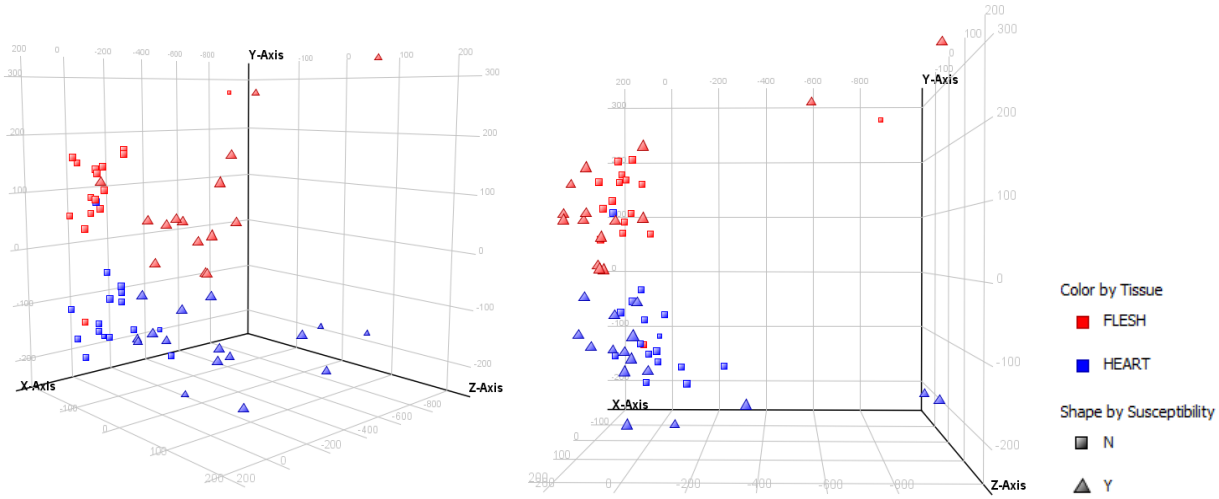


Figure 6.33 3D Principal Component Analysis scatter plot showing differences between tissues of potato stocks with different susceptibility in negative mode [Y= susceptible stock 7, N = non-susceptible stock 3) (x= 23.81%, y= 11.3%, z= 8.88%).

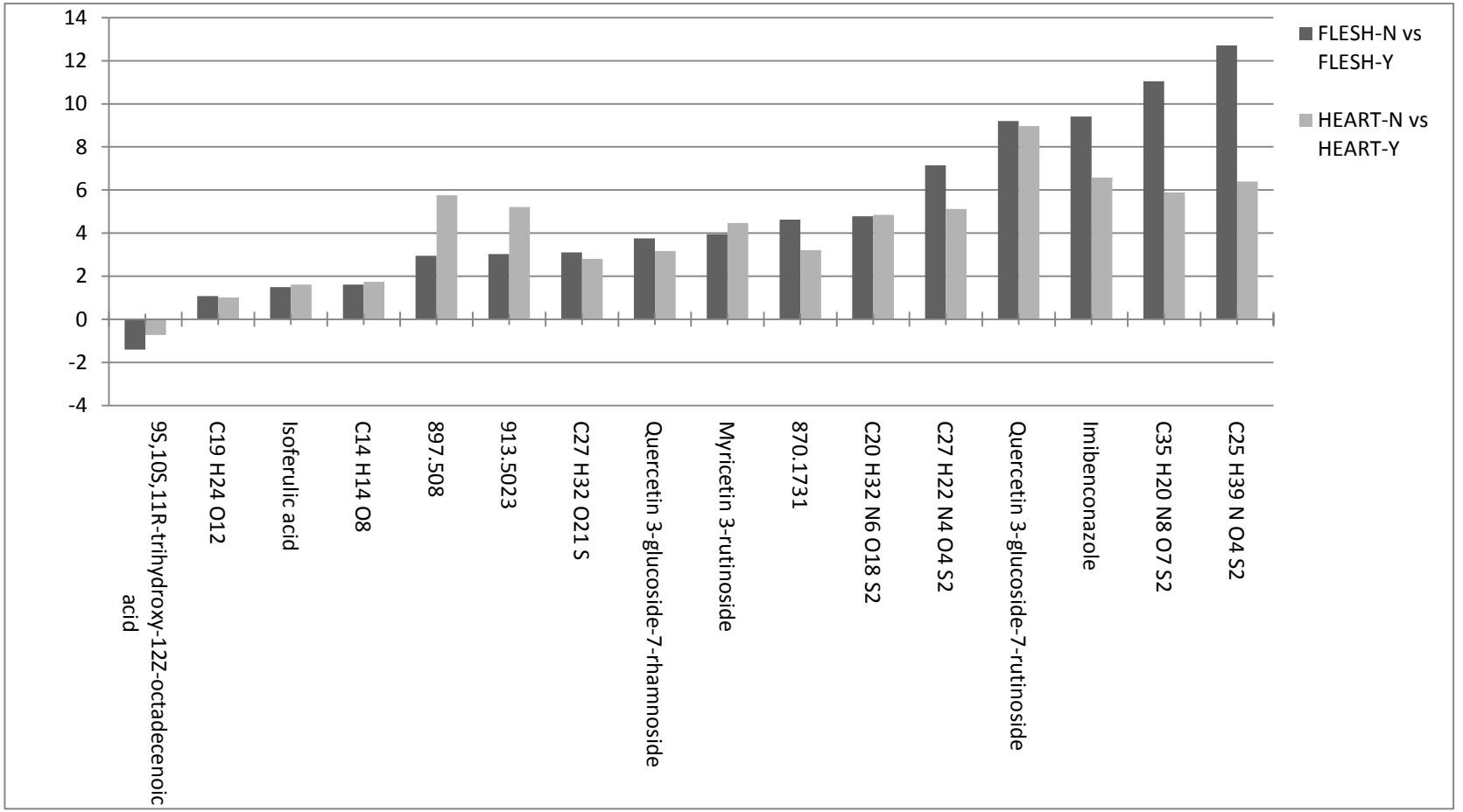


Figure 6.34 Fold-change analysis results of 'tissue vs. susceptibility' interpretation in 'experiment G'. Metabolite regulation (log FC normalized) is shown in negative mode. [Y= susceptible stock 7, N = non-susceptible stock 3) (Appendix E; Table 3.2).

Positive ionization mode

In general, 2153 known and unknown metabolites in total were detected in positive mode. Firstly, interpreting the 'susceptibility' condition 285 out of 2153 metabolites remained after filter by frequency with a coefficient of variation of less than 25%. According to Figure 6.35, almost 84% of the total variance showed on x, y and z axis and stock 7 (Y) was partially separated from stock 3 (N). Statistical analysis was performed using Moderated T-test pairing control samples of stock 7 with control samples of stock 3 resulting in 11 significant metabolites ($P < 0.05$) (Appendix E; Table 4.1). Results of fold change analysis on the same pairing indicated that five flavonoids were all down-regulated in stock 7 (susceptible to BH) (Fig. 6.36).

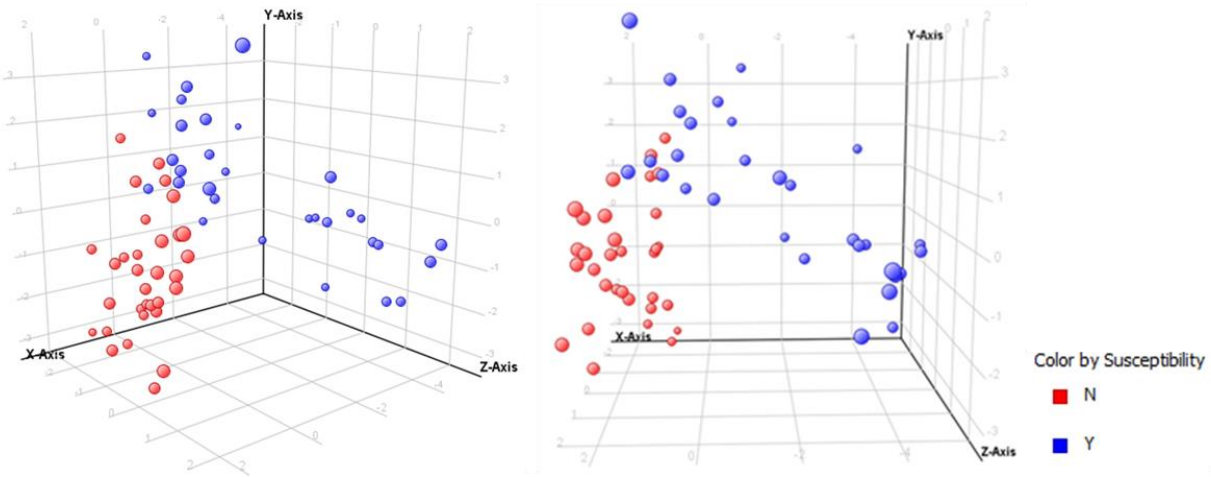


Figure 6.35 3D Principal Component Analysis scatter plot showing differences between potato stock susceptibility in positive mode [N = stock 3 (non-susceptible to BH), Y = stock 7 (susceptible to BH)] (x= 48.93%, y= 21.66%, z= 13.69%).

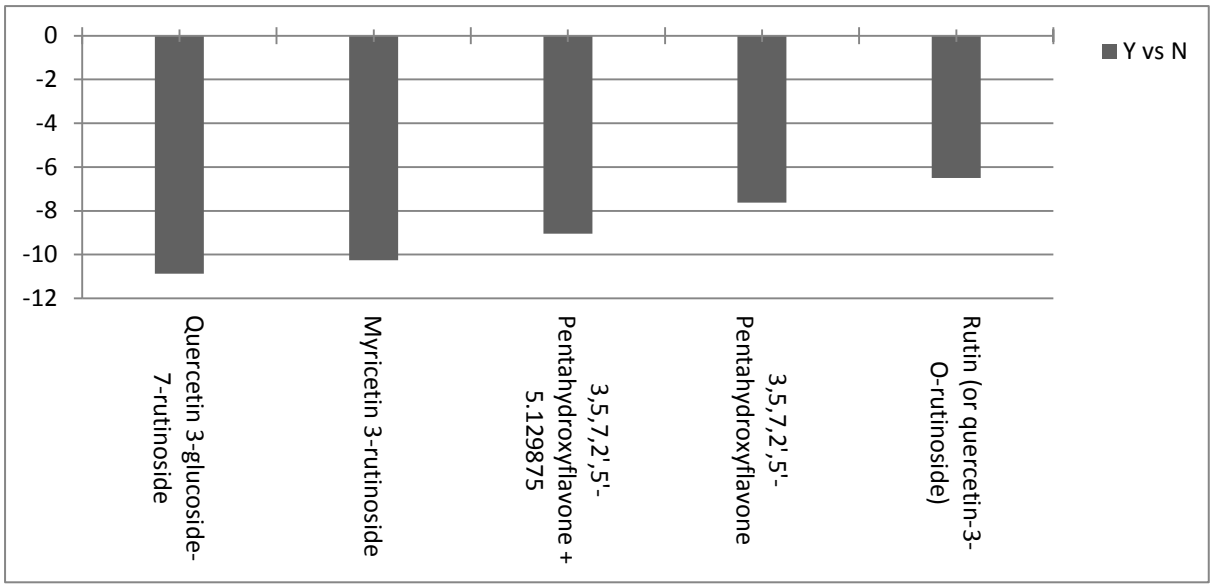


Figure 6.36 Fold-change analysis results of 'susceptibility only' interpretation in 'experiment H'. Metabolite regulation (log FC normalized) is shown in positive mode (Y = susceptible stock 7, N = non-susceptible stock 3) (Appendix E; Table 4.2).

Interpretation of 'tissue vs. susceptibility' in positive mode, filtering by frequency reduced the total number of metabolites (n = 2153) to 379 that they were additionally reduced to 82 metabolites based on a coefficient of variation of less than 10%. However, a clear separation of tuber tissue according to stock susceptibility to BH was not observed (3D scatter plot not shown) and Two-way ANOVA was not performed.

6.4. Discussion

An untargeted mass spectrometry based metabolomic approach was evaluated in order to study the metabolite variation of different tuber tissues derived from potato stocks with different susceptibility to BH disorder selected from year 1 and year 2. In general, more than 1000 and 3000 known and unknown metabolites in total were identified in negative and positive mode, respectively in year 1 analysis. In year 2 analysis, more than 2000 and 4000

known and unknown metabolites in total were detected in negative and positive ion mode respectively.

6.4.1. Metabolomic differences between affected and non-affected potato tuber tissues

According to the first part of year 1 analysis, 3D PCA scatter plots showed a good separation between those samples showing tissue discolorations with intensity BH > BC > BCL > pith > control based on a coefficient of variation of 10% in both ion modes. In terms of the general interpretation ('discoloration only'), BH samples were clearly separated from the other less intense discolorations (BC, BCL and pith) and the control. Also control and BCL were well grouped, but BC and pith were partially mixed with BCL and control. That was probably due to tissue discoloration difference as flesh tissue samples of those less intense discolorations were not discoloured. Additionally, more specifically interpretations ('tissue vs. discoloration') indicated clear separation between flesh and heart tissue samples with BH and BC discoloration when compared with the control. The other less intense discolorations (BCL and pith) did not show a very clear separation from the control samples.

However, fold change analysis results revealed a large variation in metabolite regulation among the interpretations. Generally, most of the known metabolites identified were fatty acids and also a plethora of unknown metabolites was observed. Few of the latter metabolites up regulated in discoloured tissue samples were observed. BH disorder in potato tubers may be easily distinguished due to the dark brown or black discoloration of the heart and flesh tuber tissue severity depended (Bartholomew, 1914; O'Brien and Rich, 1976; Wale *et al.*, 2008; Kumar Chaurasia, 2009), so a greater metabolite regulation was observed in those samples as it was expected. Evidence of cell membrane lipid peroxidation and tissue damage was observed. Firstly, identification of unsaturated hydroxy fatty acids such as

9S,10S,11R-trihydroxy-12Z-octadecenoic acid, 12,13-dihydroxy-11-methoxy-9-octadecenoic acid, 9-HOTE (9-hydroxy-10,12,15(E,Z,Z) octadecatrienoic acid) and 17-hydroxy-linoeic acid in BH samples indicated degradation of linoleic acid. Linoleic acid can enzymatically be converted to di-and trihydroxy fatty acids by lipoxygenases (Hamberg and Hamberg, 1996; Kim *et al.*, 2002; Gobel *et al.*, 2003). In positive mode, a metabolite was up regulated in BH heart samples and identified as 4-oxo-nonenal. This metabolite has previously been characterized as a novel lipid peroxidation product (Rindgen *et al.*, 1999; Lee *et al.*, 2000). Another interesting metabolite identified as 4-CEHC (2,5,7,8-tetramethyl-2-(2'-carboxyethyl)-6-hydroxychroman) showed an up regulation in flesh (mainly) and heart samples of BH and BC discolorations in positive mode again. It is reported that this compound is a major metabolite of a-Tocopherol the most active form of vitamin E (Schultz *et al.*, 1995; Sontag and Parker, 2002). Spychalla and Desborough (1990), reported that a-Tocopherol in potatoes has a protective action on cell membrane polyunsaturated fatty acids from lipid peroxidation under stress. An increase of a-Tocopherol was showed after storage of potato tubers for 40 weeks at low temperatures (3 and 9^{o}C) (Spychalla and Desborough, 1990). Also, the down regulation of glutathione oxidized in BH heart samples which plays an important role in cellular redox status probably indicates an impairment of the glutathione/ascorbate pathway (Dipierro and De Leonardis, 1998; Pedreschi *et al.*, 2009; Correa *et al.*, 2012). However, those findings were related with the effects of long term storage of potatoes at low temperatures and subsequent aging as lipid peroxidation occurs due to free radical build up causing cell membrane deterioration and leakage (Lojkowska and Holubowska, 1988; Spychalla and Desborough, 1990; Dipierro and De Leonardis, 1998). In addition, the fact that some of the fatty acids were present in control samples in this study indicates that the initial storage contributed to tissue cell membrane deterioration as all the samples analysed have been stored for more than 16 weeks at 1.5^{o}C.

In terms of discoloration, it has previously been hypothesized that phenolic compounds such as tyrosine, chlorogenic acid and related compounds are involved in tissue discoloration development in potato tubers affected by similar to BH physiological internal disorders such as brown centre and internal heat necrosis (Reeve, 1968). However, in this study chlorogenic acid was down regulated in BH samples indicating a possible further enzymatic oxidation to O-quinones due to tissue wounding (Pierpoint, 1966; Pantington *et al.*, 1999, Takahama *et al.*, 1997, 1999, 2004). Moreover, a relationship between more intense (BH) and less intense tissue discolorations as initiators of BH cannot be confirmed as the metabolite identification and regulation did not show a similar pattern in brown discolorations (BC, BCL and pith) when compared to control samples. Another interesting known metabolite identified was the 5-O-feruloylquinic acid (an ester of ferulic and quinic acid) which was showed to be down regulation in BH samples. This metabolite is considered a relative to chlorogenic acid as both are derivatives of quinic acid and caffeic acid. Ferulic acid (a hydroxycinnamic acid) and its esters confer cell wall adhesion and rigidity (Rechner *et al.*, 2001; Nara *et al.*, 2006; Leiss *et al.*, 2011).

The BH severity in year 2 was not as much as in year 1, as BC, BCL and pith tissue discolorations were only observed. At a first attempt, each discoloration was separately compared with control but there was poor clustering according to 3D PCA scatter plots (data not shown). However, a second attempt was carried out through merging all the discoloured samples and creating a new ‘discoloration’ condition (A: affected). On this second attempt, 3D scatter plots showed only a separation between tuber tissues regardless the discoloration in both ion modes and there were no significant differences after ANOVA performance.

6.4.2. Metabolomic differences between control samples of potato stocks with different susceptibility to blackheart disorder

In both years analysis, 3D PCA scatter plots showed a clear separation according to potato stock susceptibility to BH and indeed further separation between those tuber tissue samples from non- and susceptible stocks in some of the interpretations ('tissue vs. susceptibility') with a coefficient of metabolite variation of 10 or 25%. In general, fold change analysis revealed significant regulations of known secondary metabolites such as glycoalkaloids, flavonoids and other phenylpropanoid related metabolites. Flavonoids and steroidal glycoalkaloids both represent important groups of the secondary metabolism in plants and have extensively been studied in potatoes before (Harbone, 1959; Bostock *et al.*, 1982; Cantwell, 1996; Lewis *et al.*, 1998; Friedman *et al.*, 2006; Payyavula *et al.*, 2012, 2013).).

Glycoalkaloids are considered as the toxic group of the secondary metabolites and are stimulated by many factors such as light exposure, temperature, storage conditions and mechanical injury (Jadhav *et al.*, 1980; Cantwell, 1996; Smith *et al.*, 1996; Simonovska and Vork, 2000). A-chaconine and a-solanine are the principal glycoalkaloids in potatoes accounting ca. 95% of the total glycoalkaloids, distributed in all parts of the potato plant and their content varies in the potato tuber [(peel > cortex > flesh – pith (not detectable)]. A-chaconine and a-solanine synergism results in toxic effects (Smith *et al.*, 1996; Friedman, 2006; Mammicka, 2008). Although it has been proposed that both metabolites are synthesised via the mevalonate/isoprenoid pathway sharing the same aglycone solanidine and differ in the sugar moiety bounded to the aglycone, however, the glycoalkaloid synthesis in potatoes has not been clearly elucidated yet (Väänänen, 2007; Mandimika, 2008; Khan *et al.*, 2013).

Fold change analysis results of year 1, revealed differences in glycoalkaloid content between stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) in positive mode. A down regulation of a-chaconine was observed in those samples of stock 23 regardless the tissue. On the other hand, interpretation between tissue and susceptibility conditions showed that solanine, solanidine and solasonine (another aglycone) were up regulated in flesh control samples of stock 12. Also, solanine and solanidine were up regulated in heart tissue samples of stock 12, but solasonine did not show any changes. In addition, no changes in glycoalkaloid content in heart control samples of stock 23 were observed. According to those findings, it is unclear whether the glycoalkaloid content may be related with potato stock susceptibility to BH. Jadhav *et al.* reported that total glycoalkaloid content was increased in potatoes cvs. Russet Burbank, Norgold Russet and Pontiac with slight and severe BH and hollow heart (HH: another internal physiological disorder in potatoes) incidence due to tissue damaged caused by BH and HH, but concluded that these were less potent factor stimulating the glycoalkaloid synthesis compared to other factors as light and mechanical injury (Jadhav *et al.*, 1980). Increase in total glycoalkaloid content in potato cultivar Torrindon due to injury and tissue damage has previously been reported (Dale *et al.*, 1998). Glycoalkaloids are localized and accumulated in the vacuoles and the cytoplasm and may be transferred if the tissue is damaged (Väänänen, 2007). However, in this assessment, only control samples were analysed and yet no indications of glycoalkaloid accumulation in discoloured samples were observed in the previous assessment (see 6.4.1.). Also, no significant differences were observed between those control samples of year 2 Maris Piper stocks [stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH)]. All potato stocks in both years had initially been stored for a long period in air at 1.5^{o}C and analysed after storage at 15 and 20^{o}C in year1 and year 2, respectively. It has been reported that low storage temperature (0 – 5^{o}C) increases the glycoalkaloid content in potatoes due to stress caused (Dale *et al.*, 1998; Lawley, 2013).

However, Maris Piper stocks of each year have grown in different locations. Although it has been proposed that Maris Piper is light-insensitive cultivar, different growing conditions may affect the glycoalkaloid content in potatoes (Percival, 1999; Smith *et al.*, 1998; Sengun *et al.*, 2004).

Another two metabolites of interest were identified in positive mode. Phenylpropiolic acid showed an up regulation in those samples of stock 23 regardless the tissue. It is reported that this natural compound occurs in plant pathways and is involved in pathogen resistance (La Camera *et al.*, 2004). Interpretation between tissue and susceptibility revealed that phenylalanine which is the precursor for the phenylpropanoid pathway did not show any changes in flesh samples of stock 12 when compared with flesh samples of stock 23 and it was down regulated in heart samples of stock 12 compared to heart samples of stock 23.

Flavonoids were also identified in both years. Flavonoids are those metabolites that share the same backbone C6-C3-C6 consisted of two aromatic cycles (A and B) linked to a heterocycle (C) containing an oxygen atom and modifications (i.e. hydroxylations, glycosylations, methylations etc.) on the backbone allow the classification of those metabolites into flavonols, flavan-3-ols, flavanones, flavones, isoflavones and anthocyanins (Rice-Evans *et al.*, 1996; Pourcel *et al.*, 2006; Andre *et al.*, 2009; Pinheiro and Justino, 2012, Petrussa *et al.*, 2013). It has been reported that most of the flavonoids are present as glycosides synthesised by glycosylation namely a sugar attached to the aglyone using glycoyltransferases (Kim *et al.*, 2006, 2013; Aksamit-Stachurska *et al.*, 2008; Simkhada *et al.*, 2010). Recent work on control and transgenic potato tubers cv. Desiree using glycoyltransferase gene isolated from several cold-induced clones derived from cold-resistant potato species *Solanum sogarandinum* showed that glycosyltransferases might control the phenylpropanoid pathway (Aksamit – Stachurska *et al.*, 2008). In year 1 analysis, two

polyhydroxyflavones [(hibiscetin or 3,5,7,8-tetrahydroxy-2-(3,4,5-trihydroxy phenyl) chromen-4-one and 5,7,3',4',5'-pentahydroxy-3,6,8-trimethoxyflavone] showed an up regulation in those control samples of stock 23 (susceptible to BH) when compared with those control samples of stock 12 (non-susceptible to BH) in negative mode. Hibiscetin is a flavonol glycoside found in yellowish flower petals of *Hibiscus Sabdariffa* or roselle (Malvaceae family) an herb with antioxidant activity and functional properties (Rao *et al.*, 1941; Al-Hashimi, 2012; El-Saidy *et al.*, 2014; Obouayeba Abba Pacôme *et al.*, 2014). In negative mode still, a down regulation of the known quinic acid which is a key metabolite for chlorogenic acid synthesis was observed in stock 23. Furthermore, 4-oxoproline (involved in proline metabolism) showed down regulation in stock 23 (susceptible to BH). It has been suggested that proline may be accumulated in plants as a physiological response against to biotic and abiotic stress and might influence the adaptive responses to the stressors and its accumulation may provide protection of cell function, membrane and enzyme activity (Cheynier *et al.*, 2009).

In year 2, fold change analysis results revealed that flavonoids were up regulated in those samples of stock 3 (non-susceptible to BH). In particular, up regulation of quercetin-3-glucoside-7-rutinoside, quercetin-3-glucoside-7-rhamnoside (both diglycosides) and myricetin-3-rutinoside was observed in those flesh and heart samples of stock 3 when compared with flesh and heart samples of stock 7 (susceptible to BH) in negative mode. Similarly, quercetin-3-glucoside-7-rutinoside, myricetin-3-rutinoside, quercetin-3-rutinoside (or rutin) and two other flavonoids 3,5,7,2',5'-pentahydroxyflavone and 3,5,7,2',5'-pentahydroxyflavone + 5.129875 were all up regulated in stock 3 (non-susceptible to BH) regardless the tissue. Rutin (quercetin-3-rutinoside), myricetin-3-glucoside and similar flavonol glycosides and diglycosides identified in year 2 analysis have previously been reported in white and coloured potatoes (Lewis *et al.*, 1998; Navarre *et al.*, 2011; Payyavula

et al., 2012, 2013). Last but not least, it is worth noted that a down and up regulation of isoferulic and a trihydroxy unsaturated fatty acid (9S,10S,11R-trihydroxy-12Z-octadecenoic acid) respectively in stock 7 (susceptible to BH) was observed in negative mode. The identification of flavonoid compounds in both years and that of phenylalanine in year 1 analysis suggest differences in gene expression and regulation of the phenylpropanoid compounds and their biosynthetic pathway. However a conclusion of how these metabolites may differentiate the potato susceptibility to BH cannot be achieved based only on this data.

6.5. Conclusions

This study indicated that through an untargeted metabolomic approach it is possible to provide evidence about the involvement of number of interesting metabolites identified and to have a better understanding of the metabolite regulation. It was possible to separate tuber tissue discolorations from the control with the former tissue samples showing a greater metabolite regulation in year 1 analysis. However in year 2 analysis there were no significant differences in terms of tissue discoloration. Thus, it cannot be concluded that less intense brown discolorations are initiators of BH development. On the other hand, it seems plausible that the flavonoids identified could be used to differentiate potato stocks with different susceptibility to BH in both years and this group of phenylpropanoid compounds warrant further investigation.

CHAPTER SEVEN:
Feasibility study into the gas diffusivity measurement of potato stocks with different susceptibility to blackheart disorder

7.1. Introduction

Gas exchange in fruit and bulky organs is essential for metabolic processes and is caused by differences in gas concentrations between the applied external atmosphere and the internal atmosphere of plant tissues. Gas diffusion is the primary mechanism of gas exchange and is determined by many factors as the physiological status of the commodity and its respiratory activity, tissue permeability and also those barriers and pathways that gases need to follow within a tissue (Corey and Tan, 1990; Verboven *et al.*, 2008; Ho *et al.*, 2006b, 2010).

In potato tubers, O_2 diffusion initially occurs through the lenticels of the skin that are the dominant barriers, then passes through the flesh to the intercellular spaces where eventually the respiration takes place in the cytoplasm-mitochondria, while CO_2 is released following the opposite path (Wigginton, 1973; Banks and Kays, 1988; Weber, 1990; Geigenberger *et al.*, 2000; Ho *et al*., 2010). It is believed that gas-filled intercellular spaces pose the main pathway for gas diffusion. Since very early on, it was stated that tuber tissue contains small intercellular spaces showing some connectivity to one other (Devaux, 1891). Later, Woolley (1962) also reported after microscopic observations that the intercellular spaces of potatoes cv. Russet were almost all gas-filled with a diameter of 10-15 μm showing a connectivity path of 0.5 mm (ca. 3 to 6 cells diameters). Nevertheless, in contrast with other bulky crops, for instance apples and pears, potato tuber is more compact having low porosity with only 1-2% of intercellular space volume in the tissue (Banks and Kays, 1988; Scotsmans

et al., 2003; Ho *et al.*, 2006). Also, it has previously been reported that adequate O_2 concentration is contained in the intercellular spaces of the tuber tissue due to low respiration rate of the tuber under regular storage conditions, but partial anoxia might occur at the centre of the tuber under adverse environments (Burton, 1950; Woolley, 1962; Wigginton, 1973; Abdul-Baki and Solomos, 1994). Insufficient gas exchange and limited supply of O_2 might lead to hypoxic or anoxic atmospheres resulting in physiological changes and tissue cell impairment and yet cell death (Geigenberger *et al*., 2000; Ferreira de Souza *et al*, 2002; Verboven *et al.*, 2008; Zabalza *et al.*, 2009).

Early studies related to gas diffusivity of the potato were mainly focused on the gas composition of intercellular spaces or on its influence on sprouting. Several methods that have previously been used estimating the internal gas composition of tuber tissue showed accuracy limitations due to low porosity of the tuber making their estimations difficult (Devaux, 1891; Magness, 1920; Thornton, 1939; Gorter and Nadort, 1941; Burton, 1950, 1968; Woolley, 1962). Later, other publications referred to gas diffusion through the potato skin and flesh (Wigginton, 1973; Banks and Kays, 1988; Abdul-Baki and Solomos, 1994). Currently, there is a dearth of information on estimating the gas diffusivity of different and specific regions of the potato tuber. Since it has been reported that blackheart disorder (BH) in potato tubers is induced when O_2 is depleted and consumed faster than may be supplied resulting in cell necrosis mainly in the central part of the tuber (Lipton, 1967; Smith, 1978; Banks and Kays; 1988), thus it would be interesting to estimate the O_2 diffusion in specific regions of the perimedullary and the central pith.

For the first time using a gas diffusion model set up combined with optical sensors (Ho *et al.*, 2006a), this study aimed to investigate the O_2 diffusivity in flesh and heart tissue discs of potato stocks cv. Maris Piper with different susceptibility to BH. That same set up has extensively been used to elucidate changes in gas diffusivities of pear fruit with or without

core breakdown disorder (Ho *et al.*, 2006a,b 2007). As the gas exchange mechanism is largely dependent on the intercellular space and cell structural arrangement, three dimensional (3D) images using x-ray micro Computed Tomography (micro-CT) were obtained in order to visualize and better estimate the pore formation of different tuber tissues (flesh and heart). X-ray scanning has previously been used to detect hollow heart disorder in potatoes (Harvey, 1937; Nylund and Lutz, 1950; Finney and Norris, 1978; Watts and Russell, 1985). Later, x-ray Micro-CT was performed to assess the induction of common scab disease in potato roots (Han *et al.*, 2008) and also growth velocity of potato tuber was determined using comparative transcriptome analysis coupled with micro-CT (Ferreira *et al.*, 2010).

7.2. Materials and methods

Sample preparation before x-ray micro-computed tomography scanning and sample preparation for potato tuber volume, density and respiration rate measurement are described in Chapter 3; Section 3.7.1, 3.7.2 and 3.7.3, respectively. Sample preparation for O_2 diffusivity measurement and diffusion set up system are also described in Chapter 3: materials and methods (see Section 3.7.4 and 3.7.5, respectively).

7.3. Results

7.3.1. Example of micro-Computed Tomography (micro-CT) scans and three-dimensional (3D) images

Example of flesh and heart tuber tissue micro-CT scans (two dimensional – 2D) of stock 4 (non-susceptible to BH) and stock 10 (susceptible to BH) are shown in the figures below (Figs. 7.1 - 7.4). Micro-CT scans of flesh tissue of tuber 2 from stock 10 are not shown

due to poor scanning resolution. The images were segmented into cells, intercellular spaces and also starch granules based on a greyscale threshold. Intercellular spaces and starch may easily be distinguished as black hole shapes and light grey coloured granules, respectively. According to Figures 7.1 to 7.4 a variation in size and shape of the intercellular space it was shown. It was also observed that starch granules were more accumulated next to the intercellular spaces regardless of the tissue examined. It is worth noting that imaged potato tubers were initially stored at 3.5^{o}C for ca. 8 months before analysis. Differences in starch accumulation in flesh and heart samples of stock 4 (non-susceptible to BH) compared to those samples of stock 10 (susceptible to BH) were observed. Starch tended to be more accumulated in flesh than in heart tissue of stock 4 (Figs. 7.1 and 7.2). Starch accumulation was similar in the flesh and heart samples of stock 10 (Fig. 7.3 and 7.4).

3D image reconstruction is based on a set of those 2D scans obtained after x-ray micro-CT scanning. Example of 3D images of flesh and heart tuber tissue of stock 4 (non-susceptible to BH) and stock 10 (susceptible to BH) are shown in Figures 7.5 and 7.6 respectively. Regarding the intercellular space formation a large variability was observed among flesh and heart tuber tissue. That was due to the ‘noise’ caused by starch accumulation and also the sampling position of the potato tuber from which flesh and heart tissues were cut and scanned.

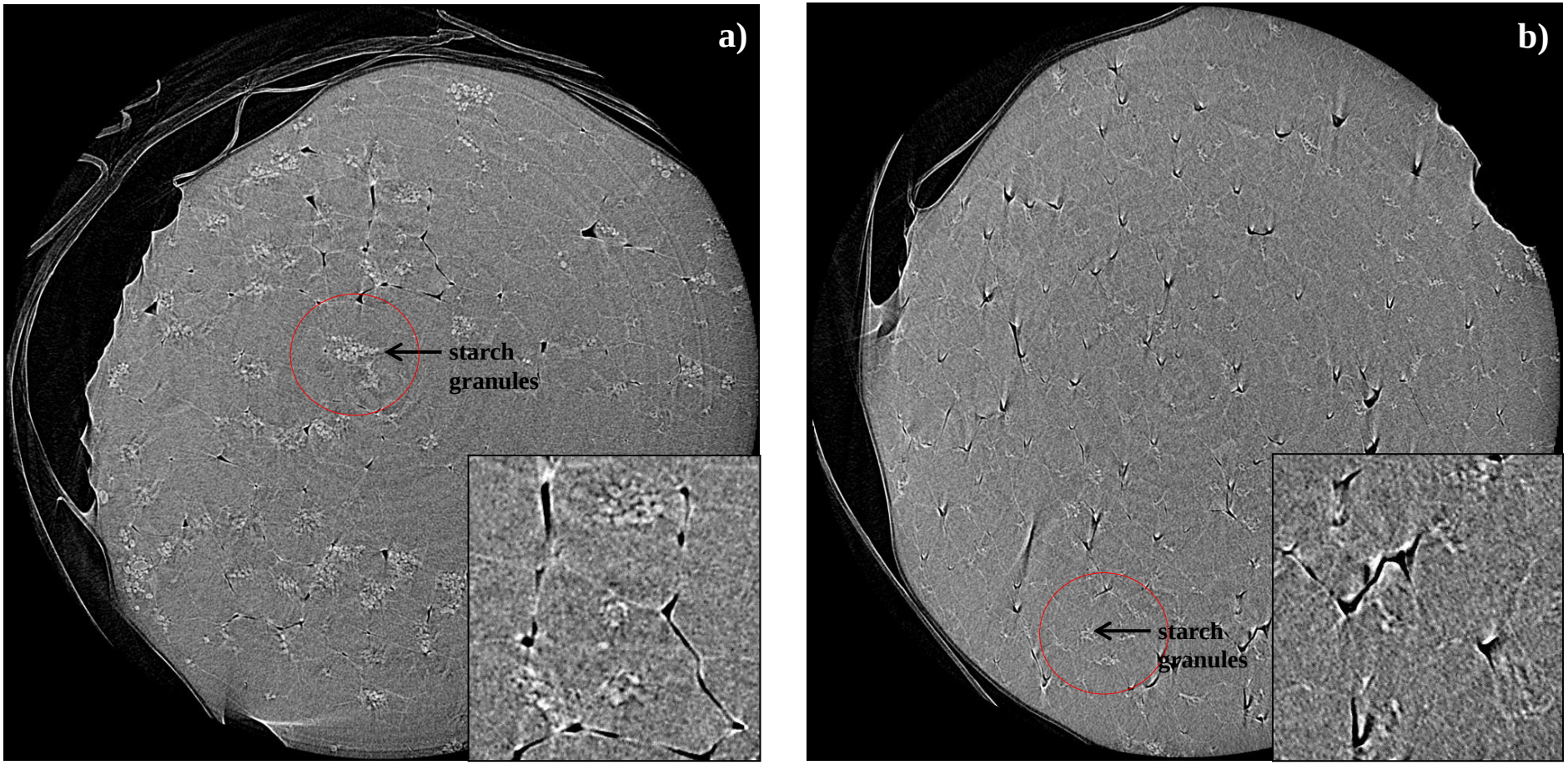


Figure 7.1 X-ray micro-CT scans of flesh (a) and heart (b) tissue (3 mm in diameter and 5 mm in thickness) of tuber 1 from stock 4 (non-susceptible to BH) at 2 μm pixel resolution. Intercellular spaces are shown as a black hole shape. Suspected starch granules are shown in light grey colour. Selected regions of interest (500 x 500 pixels) are shown.

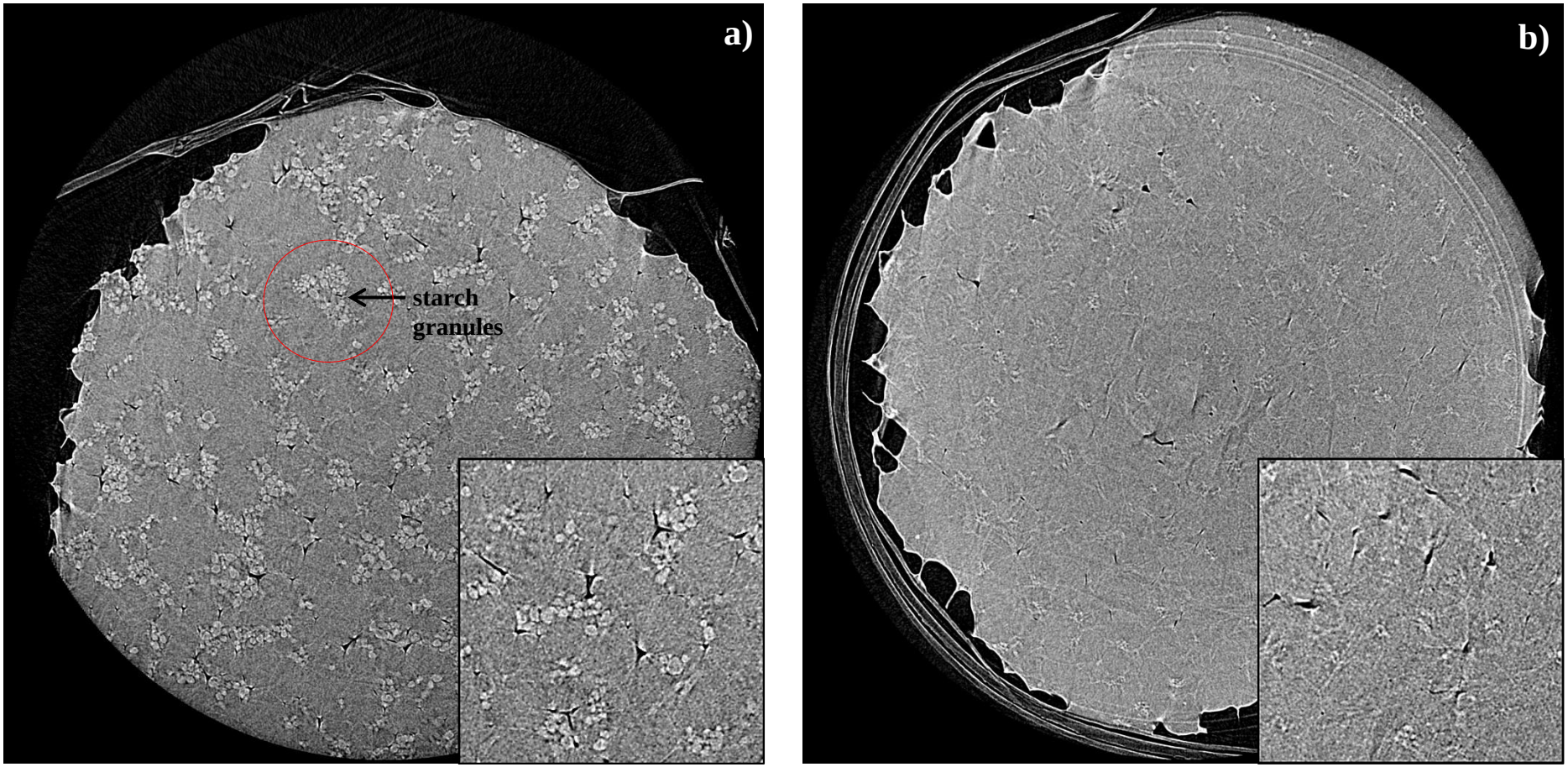


Figure 7.2 X-ray micro-CT scans of flesh (a) and heart (b) tissue (3 mm in diameter and 5 mm in thickness) of tuber 2 from stock 4 (non-susceptible to BH) at 2 μm pixel resolution. Intercellular spaces are shown as a black hole shape. Starch granules are shown in light grey colour. Selected regions of interest (500 x 500 pixels) are shown.

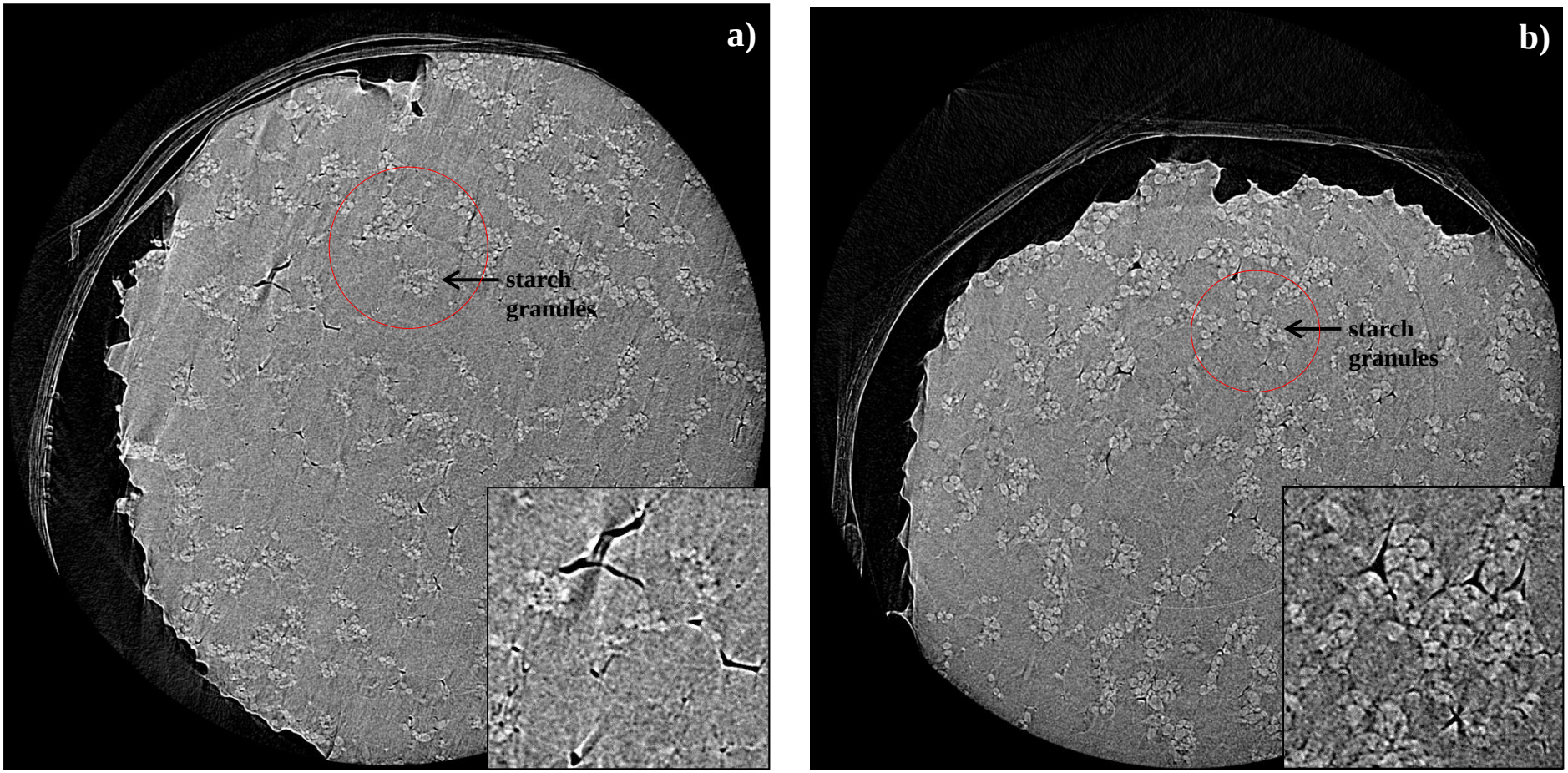


Figure 7.3 X-ray micro-CT scans of flesh (a) and heart (b) tissue (3 mm in diameter and 5 mm in thickness) of tuber 1 from stock 10 (susceptible to BH) at 2 μm pixel resolution. Intercellular spaces are shown as a black hole shape. Starch granules are shown in light grey colour. Selected regions of interest (500 x 500 pixels) are shown.

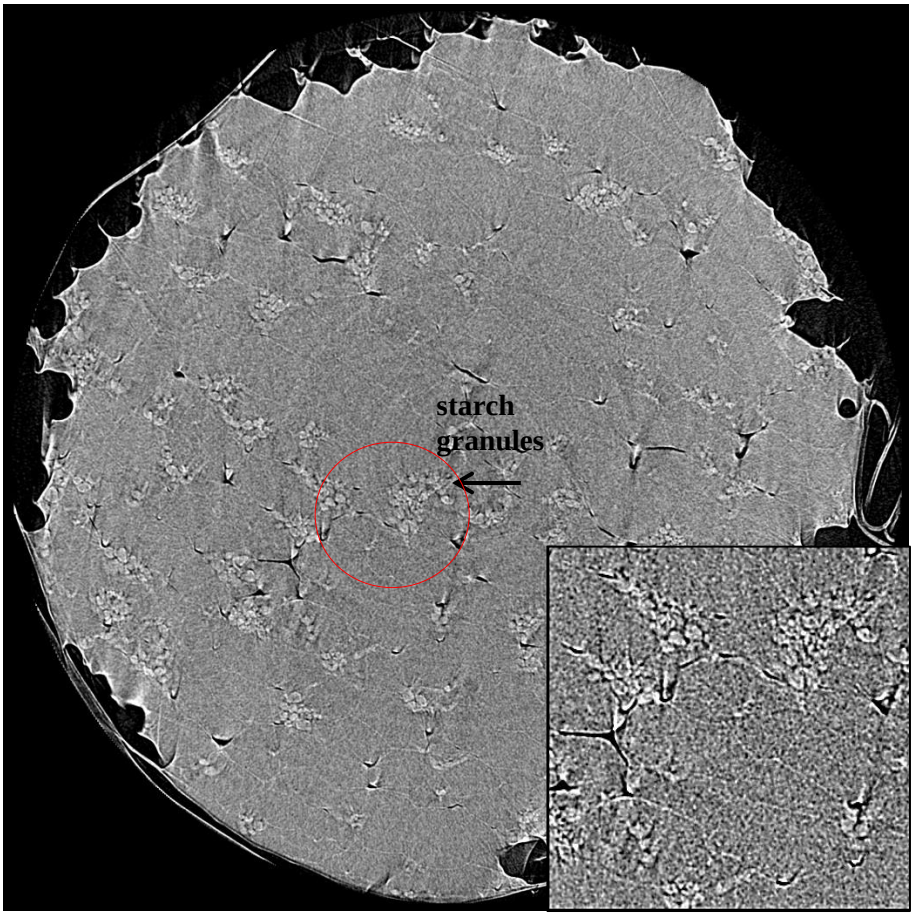


Figure 7.4 X-ray micro-CT scan of heart tissue (3 mm in diameter and 5 mm in thickness) of tuber 2 from stock 10 (susceptible to BH) at 2 μm pixel resolution. Intercellular spaces are shown as a black hole shape. Starch granules are shown in light grey colour. Selected regions of interest (500 x 500 pixels) are shown.

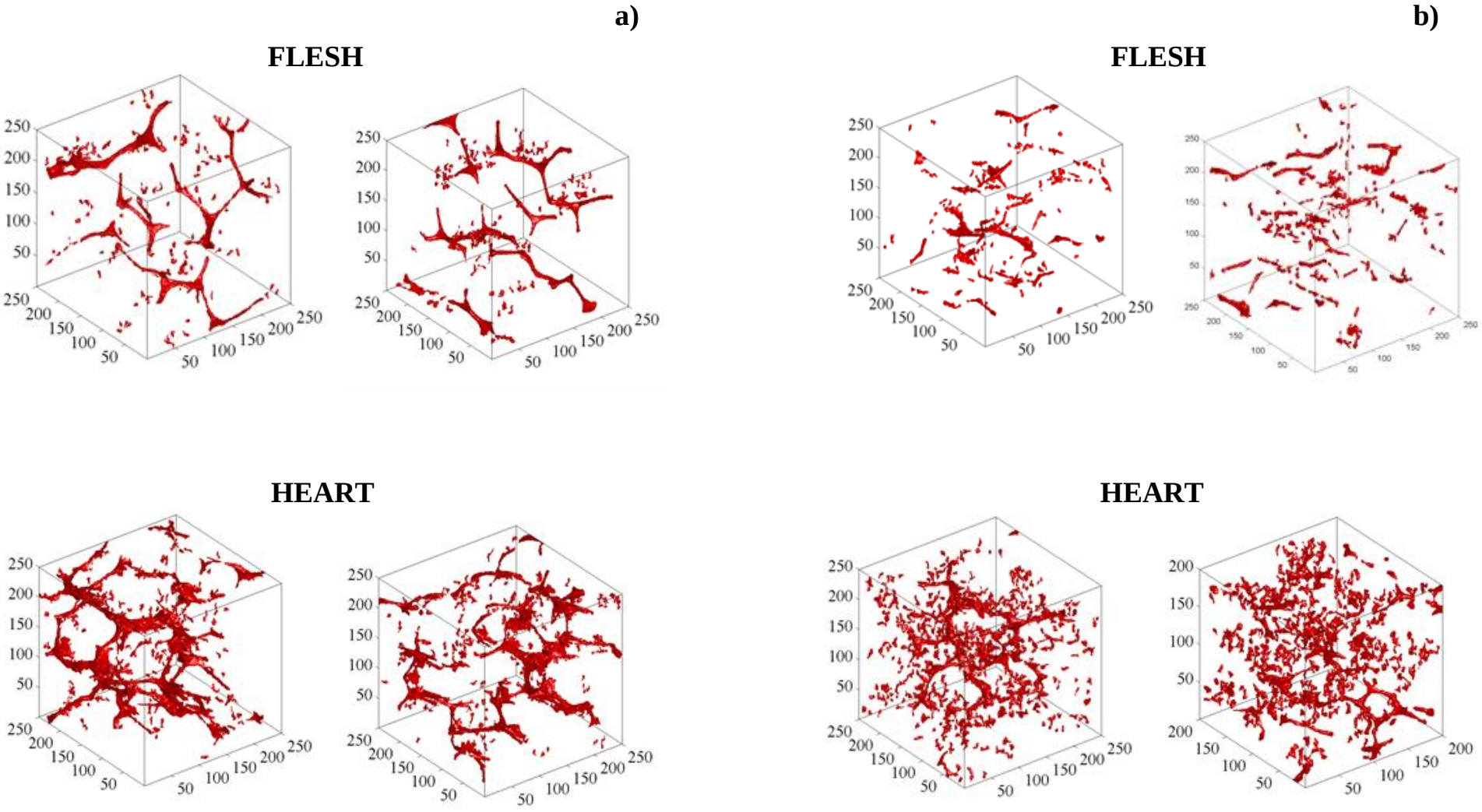


Figure 7.5 3D microstructure of flesh and heart tissue of tuber 1 (a) and tuber 2 (b) from stock 4 (non-susceptible to BH) reconstructed after micro-CT scanning.

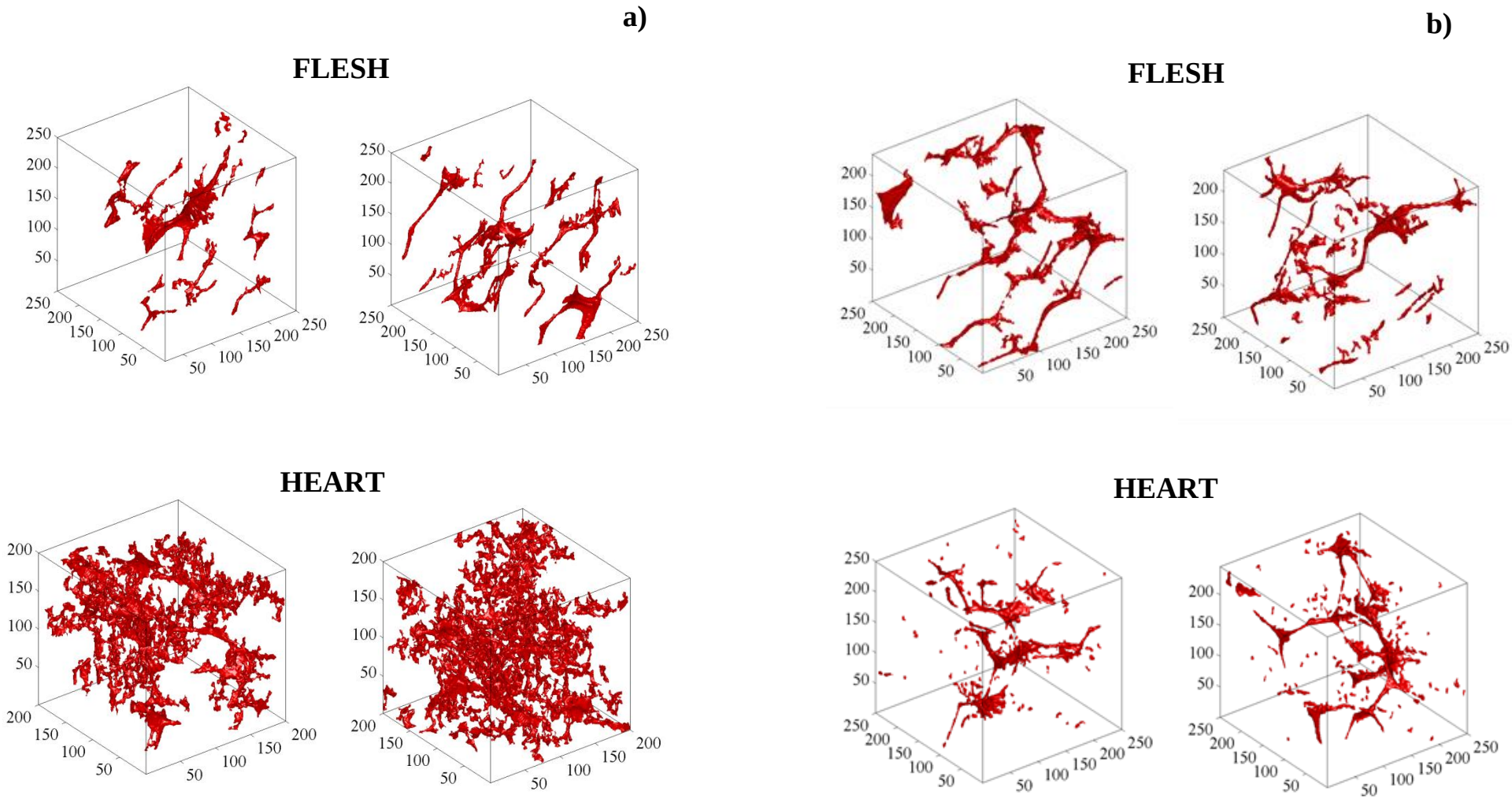


Figure 7.6 3D microstructure of flesh and heart tissue of tuber 1 (a) and tuber 2 (b) from stock 10 (susceptible to BH) reconstructed after micro-CT scanning.

7.3.2. Oxygen diffusivity

Oxygen diffusivity (D_{O2}) of flesh and heart tissue of stock 10 (susceptible to BH) and stock 4 (non-susceptible to BH) was calculated using COMSOL Multiphysics® with MATLAB® Scripting software. The results were averaged (n = 8 / sample / stock) and tabulated in Table 7.3. During this experiment all tuber tissue samples were sound with no indications of discoloration except a brown centre (BC) discoloration that was surprisingly discovered in that tuber of stock 4 (non-susceptible to BH) at measurement on day 7 (Fig. 7.7). According to Table 7.1, it was shown that the highest (1.08×10^{-9} m^2 s^{-1}) and the lowest (0.5×10^{-9} m^2 s^{-1}) average O_2 diffusivity was observed in the heart and flesh tissue of stock 10 (susceptible to BH), respectively. O_2 diffusivity of heart tissue of stock 4 was slightly higher than that of flesh tissue (0.74×10^{-9} and 0.69×10^{-9} m^2 s^{-1} respectively). Also, the O_2 diffusivity of that heart tissue of stock 4 with BC discoloration was 0.069×10^{-9} m^2 s^{-1} (Table 7.1).

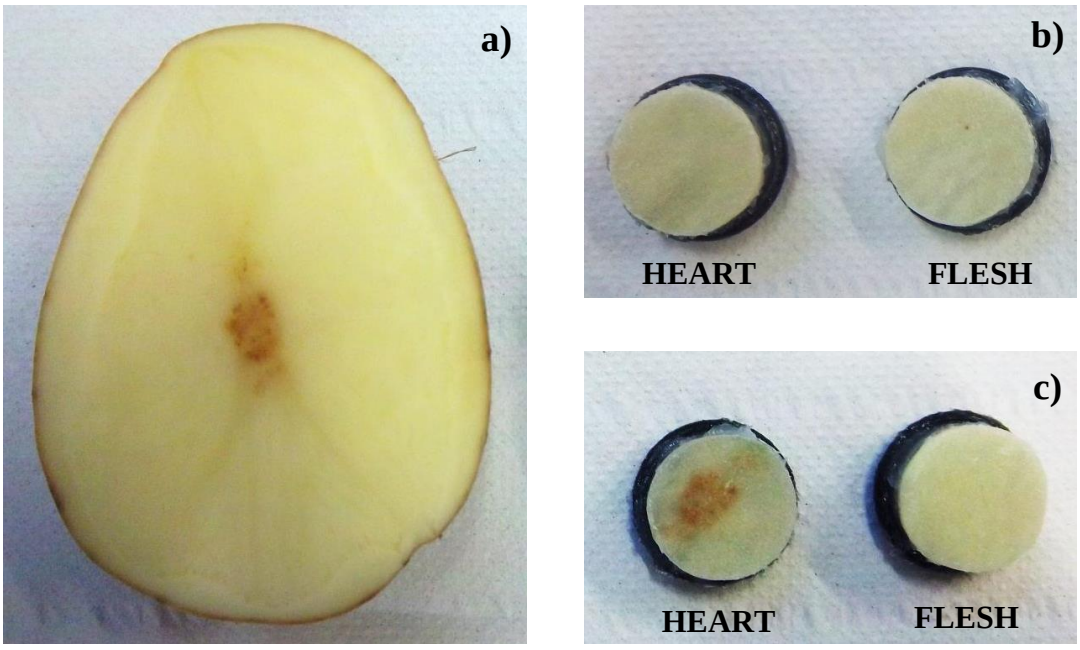


Figure 7.7 BC (brown center) discoloration in tuber of stock 4 (non-susceptible to BH) (a). Heart and flesh tissue samples of stock 10 (susceptible to BH) (b) and stock 4 (c) before O_2 diffusion measurement on day 7.

Table 7.1 Oxygen diffusion [D_{O2} (m^2 s^{-1})] of flesh and heart tissue from stock 10 (susceptible to BH) and stock 4 (non-susceptible to BH).

Measurement	Stock 10		Stock 4	
	FLESH	HEART	FLESH	HEART
1	0.095×10^{-9}	2.74×10^{-9}	0.080×10^{-9}	0.158×10^{-9}
2	0.105×10^{-9}	2.70×10^{-9}	4.81×10^{-9}	0.094×10^{-9}
3	3.21×10^{-9}	0.119×10^{-9}	0.079×10^{-9}	0.090×10^{-9}
4	0.196×10^{-9}	0.172×10^{-9}	0.087×10^{-9}	0.131×10^{-9}
5	0.083×10^{-9}	2.57×10^{-9}	0.110×10^{-9}	5.07×10^{-9}
6	0.075×10^{-9}	0.081×10^{-9}	0.087×10^{-9}	0.069×10^{-9}
7	0.162×10^{-9}	0.142×10^{-9}	0.173×10^{-9}	0.069×10^{-9}
8	0.070×10^{-9}	0.126×10^{-9}	0.095×10^{-9}	0.107×10^{-9}
Average	0.5×10^{-9}	1.08×10^{-9}	0.69×10^{-9}	0.74×10^{-9}

7.4. Discussion

The results revealed large variation of O_2 diffusivities in different tuber tissue samples of cv. Maris Piper potato stocks with different susceptibility to BH among the micro-CT scans. As gas-filled intercellular spaces are believed to pose the main pathway for gas diffusion, x-ray micro-computed tomography was initially used for a better visualization of the tissue structure in flesh and heart tuber tissue of stock 10 (susceptible to BH) and stock 4 (non-susceptible to BH). According to micro-CT scans tissue cells could not be easily distinguished. However, accumulated starch granules and gas-filled intercellular spaces were clearly visualized. Differences in starch accumulation between the tuber tissues were observed, but those differences cannot be compared between stock 10 (susceptible to BH) and stock 4 (non-susceptible to BH) due to small sample replication (n = 2 samples / tissue / stock). Both micro-CT scans and 3D images showed that the gas-filled intercellular spaces of flesh and heart tissue were not well connected and varied in size and shape. Also, the intercellular space visualization was negatively influenced from starch granule accumulation

by creating ‘noise’ on the 3D image quality. A greater sample replication it would be more useful to better distinguishing the starch accumulation and yet to better estimate possible differences of intercellular space formation between specific regions of the tuber tissue. For the micro-CT scanning, the flesh tissue was derived from the perimedulla region and the heart tissue from the medullary regions (pith). Those two tuber regions differ because of the tissue cell structure as parenchyma cells of the perimedulla (flesh) are bigger and more starch abundant than of those cells present in the medullary zone (pith) and thus this difference in starch abundance may be responsible for that wet-translucent looking of the pith (Reeve *et al.,* 1969; Sadowska *et al.,* 2007). Microscopic analysis of flesh and pith cellular structure that has extensively been conducted in different Polish potato cultivars is in agreement with this size difference of tuber cells. The authors also showed some cell structure differences between those cultivars examined (Konstankiewicz *et al.*, 2002; Gancarz *et al.*, 2007, 2014). This might suggest that due to differences in cell structure, different intercellular space connectivity may be formed influencing further the gas diffusion within a tissue. Therefore, the gas-filled intercellular spaces might partially be filled up with water or sap posing an additional resistance to gas diffusion as the cell walls becoming hydrated. It has been previously hypothesized that CO_2 diffusion has greater solubility in water and transports faster in the liquid phase while O_2 mainly diffuses through the gas phase (Woolley, 1962; Himmelblau *et al.,* 1965; Abdul-Baki and Solomos, 1994; Ho *et al.*, 2006a, 2007, 2011; Licausi and Perata, 2009).

From this study, it is suggested that the discontinuity of the gas-filled intercellular spaces may be a factor governing large variation in O_2 diffusivities between flesh and heart tuber slices of both stocks analyzed. Also, some very large values of O_2 diffusivity measured might be explained due to possible gas leakage in the measurement chamber of the gas diffusion model set up used. Ideally, a lower O_2 diffusion in heart tissue of stock 10

(susceptible to BH) it would be expected. However, in this experiment results showed that average O_2 diffusivity of the heart tissue of stock 10 was the highest (1.08 x 10^{-9} m^2 s^{-1}). The fact that all the flesh and heart samples of stock 10 were sound without any indication of discoloration did not allow a comparison with those samples of stock 4 (non-susceptible to BH) as the O_2 diffusivities varied the same. During the whole experiment, there was only one heart sample which showed BC discoloration and interestingly this was derived of stock 4 (non-susceptible to BH), but that did not influence the results. Therefore, a further measurement of CO_2 diffusivity it would be very useful to better understand the gas diffusion in those flesh and heart tuber tissues examined. Early studies on CO_2 diffusivity in potato cv. Russet Burbank showed that the peel had a lower CO_2 diffusivity when compared to the flesh (0.06-0.07 x 10^{-9} and 25-26 x 10^{-9} m^2 s^{-1} respectively) (Abdul-Baki and Solomos, 1994). Also, gas diffusion studies on pear fruit also showed that gas diffusivities of the skin were lower than of those in the inner cortex (Lammertyn *et al.*, 2001; Schotsmans *et al.*, 2003; Ho *et al.*, 2006a). In more recent work which is partially related to this study, Ho *et al.*, 2006b studied the gas diffusivities of healthy and affected pears with core breakdown disorder induced under CA storage conditions. The authors compared flesh tissues of healthy pears versus affected pears showing that average O_2 and CO_2 diffusivities of affected flesh tissues with brown discoloration (0.185 x 10^{-9} and 0.142 x 10^{-9} m^2 s^{-1} respectively) were lower compared to sound tissues (0.246 x 10^{-9} and 0.158 x 10^{-9} m^2 s^{-1} respectively). They also concluded that there were no differences in gas diffusivities between healthy and sound pear tissues due to large variability observed between those samples analyzed.

7.5. Conclusions

The measurement of O_2 diffusivity in flesh and heart tuber tissue samples of potato stocks with different susceptibility to BH (one susceptible and one non-susceptible) was carried out using a gas diffusion measurement model set up coupled with optical sensors for the first time. To better visualize the intercellular spaces in flesh and heart tissue of both potato stocks x-ray micro-CT scanning and 3D images were used. Both micro-CT scans and 3D images showed a variation in the structure and connectivity of the intercellular spaces in tuber tissues. There was a large variation in O_2 diffusivities and it is suggested that this may be due to the discontinuity of the gas-filled intercellular spaces. Also, starch granule accumulation was considered as diffusion in liquid posing an additional barrier of the diffusion. Due to the large variability, no differences between flesh and heart tissue were observed. Furthermore, the fact that there were no indications of discoloration in those samples of the susceptible to BH stock, a comparison in stock susceptibility was not possible. Also, to gain a better understanding of the gas diffusivity at different tuber tissues the measurement of CO_2 diffusivity would be useful.

CHAPTER EIGHT:

General discussion

Consumer's demand for nutritious, tasty and secure food has become topic of considerable recent interest. The potato is a high value crop and one of the most important staple foods worldwide and is widely produced and consumed in the UK. In recent years, potato blackheart disorder (BH) has been considered as a significant cause of storage losses (Kumar *et al.,* 2009) the prevalence of which has been increasing in the UK potato industry. BH is a non-pathogenic disorder resulting in an internal brown to black discolouration mainly in the medullary tuber tissues (pith) and rarely formation of cavities and it is associated with O_2 depletion and/or CO_2 accumulation. From the consumer's point of view, good potato quality is determined by firmness, smoothness, no indications of defects and sprouts and unfavourable colours thus being aesthetically appealing enchasing consumer's confidence. On the other hand, BH disorder as for similar internal physiological disorders induced in potatoes show no external symptoms and only becomes apparent when tubers are sliced open. Thus, BH is considered as a particular problem for the fresh potato industry because quality control (QC) procedures cannot adequately check for the defect and this failure is undermining consumer confidence. To date, it is still believed that BH can develop at any temperature when O_2 supply is insufficient and may either be developed by tuber exposure to high temperatures (> 35^oC) without O_2 deprivation (Hiller, 2002; Kumar Chaurasia, 2009) or at low or very low temperatures because under those conditions gas diffusion is restricted (Stewart and Mix, 1917; Link *et al.*, 1932; Lipton, 1967; O'Brien and Rich, 1976; Smith; 1978; Hooker, 1981; Wale *et al.,* 2008). However, the storage temperature and exposure time in which BH is initiated and reaches the maximum levels of incidence cannot be predicted and it is unclear what pre- and/or post-harvest factors are involved in BH development. The

aim of this project was to study the physiological and metabolomic mechanisms involved in BH disorder by selecting potato stocks (cv. Maris Piper) with different susceptibility to BH and to have a better understanding of the factors which contribute to the development of BH disorder in order to alleviate this problem for the UK fresh potato industry.

8.1. Effect of storage temperature and shelf-life conditions on the BH incidence of potato stocks with different susceptibility to BH

According to customer complaints, BH disorder in potato tubers is more evident by the end of the winter peaking in spring time. BH becomes apparent only when tubers are sliced open namely during cooking preparation. It is assumed that inappropriate storage and shelf-life conditions and probably the packaging material itself may contribute to BH development. In this project, an attempt to mimic the shelf-life storage was evaluated by storing potato cv. Maris Piper stocks with different susceptibility to BH at a very low storage temperature and then tuber subsamples transferred at a warmer temperature and stored in air and/or under various gas combinations using the CA system. Maris Piper cultivar is the dominant main-crop produced in the UK (accounting for 15% of UK plant area) with various culinary uses.

In year 1 (2011-2012) less and more intense brown tissue discolorations localized in the central pith part of the tuber were mostly indicated in both susceptible to BH stocks (stock 20 and stock 23) starting in December 2012 after 8 weeks of storage at very low temperature (1.5 or 3^{o}C) peaking after a few months during springtime. However, the total percentage of BH incidence was quite low (< 10%) corresponding to the total number of tubers used. Stock 23 had significantly (ca. 3-fold) greater BH incidence compared to stock 20 and yet BH-like symptoms namely a dark brown to blackish discoloration were found in just three tubers of stock 23. Furthermore, no indications of discoloration were observed at baseline where no

initial cold storage temperature occurred (November 2011). That might suggest that the initial cold storage at very low temperature after baseline may trigger the brown tissue discoloration. In a similar study on storage trials of 15 Maris Piper stocks (including stock 20, 23 and 12 used in this present study) conducted by A. Briddon at Sutton Bridge Crop Science Research (SBCSR) in 2011, it was reported that BH symptoms were not detected directly after storage at very low temperatures (1.5 and 3.5^{o}C). However, he also reported that stock 23 (susceptible to BH) had ca. 6-fold higher BH incidence compared to stock 20 (susceptible to BH) and stock 12 (non-susceptible to BH) after packaging trials (Adrian Briddon, personal report, SBCSR). In year 2 (2012-2013) the incidence of BH in both experiments conducted was also quite low proportionally to the total number of tubers used. No significant differences in BH incidence were shown over storage time and yet between those susceptible to BH stocks 7 and 12. Brown tissue discolorations were only identified. Differences in BH incidence between year 1 and year 2 might be explained due to different growing seasons and growth conditions that all potato stocks analysed have been grown at (Davies, 1998).

Also, it is well known that when potatoes are transferred from a cooler to warmer temperature condensation occurs on their skin surface due to temperature difference and a restriction in O_2 diffusion arises due to the water film formed on their skin (Burton and Wigginton, 1970; Hooker, 1981; Pringle *et al.*, 1996, 2009; Wale *et al.*, 2008). So, it is worth mentioning that when potatoes were transferred unwashed from the cold temperature in order to be sampled on day 0 at 15 or 20^{o}C (in year 1 and year 2 respectively), a prior 3 - 5 hour warming up for the tubers was required before sampling. However, during this project condensation was not observed.

It has been reported that BH development is caused by gaseous differences and can occur at any temperature by O_2 deprivation (Stewart and Mix, 1917), but at low temperatures (5^{o}C) its development requires a longer time. However, it is believed that the disorder may rapidly

develop at extreme high (> 35^{o}C) or very low (0-2.5^{o}C) temperatures (Batholomew, 1916; Link *et al.*, 1932; O'Brien and Rich, 1976; Smith, 1978; Hooker, 1981; Wale *et al.*, 2008; Kumar Chaurasia, 2009). Lipton (1967) reported that incidence of BH (10 - 13%) occurred in packed potatoes cv. White Rose after storage in very low O_2 concentrations (0.5 and 1% O_2) at 5 and 15^{o}C and no indications of BH in storage at 5 and 21% O_2 (air) at higher temperatures were observed. Furthermore, Butchbaker *et al.* (1967), reported severe incidence of BH in unwashed packed potatoes cv. Kennebec after storage in CA chambers with 10% O_2 and 10-24% CO_2 at 4^{o}C for six months. After packaging trials on 15 Maris Piper stocks conducted in 2011, A. Briddon reported that BH incidence was 2-fold higher in punched polythene packed potatoes compared to those unpacked after storage at 20^{o}C for 13 days. He also concluded that initial storage temperature at 1.5^{o}C effectively increased BH incidence mainly in packed potatoes compared to 3.5^{o}C, but BH symptoms were accompanied with grey diffusion (suggested as total % of BH symptoms) were more evident in unpacked tubers (Adrian Briddon, personal report, 2011). Those findings suggest that packaging combined with storage at lower O_2 levels and/or high CO_2 concentrations, seems to be a significant barrier by further promoting the O_2 restriction (Beaudry, 2000; Fonseca *et al.*, 2000; Watkins, 2000). In this project, it might be said that O_2 was not restricted in those chambers used in both years where tubers were respiring normally and respiration rate recorded by all stocks was affected mainly from the initial cold storage temperature, the efflux of CO_2 for those tubers held under CA conditions or a combination of both factors. Hooker (1981) suggested that at 1% O_2 or lower levels at 14^{o}C or higher temperatures will cause stimulation of anaerobic respiration, greater membrane permeability with result to decay and BH concluding that these effects might not be evident at lower temperatures.

BH symptoms are linked with black discoloration localized in the very central part of the tuber (pith), but sometimes may diffuse in the unaffected perimedulla tuber area without

reaching the cortex, depended on the severity (Hooker, 1981). However, there is still a confusion of which are the real symptoms that govern the disorder. Kumar Chaurasia (2009) characterized the BH symptoms as a dark grey to black-purplish discoloration and Hooker (1981) reported that black to black-blue irregular discolorations were indicated in the central part of the tuber. On the other hand it seems that exclusion of O_2 leads to irregularities in shape discoloration across the pith, but at very high temperatures the discoloration may be formed in a circle-like shape (Stewart and Mix, 1917; Wale *et al.*, 2008). As previously mentioned, in this project, less and more intense brown tissue discolorations localized in pith tuber area were mostly identified and sometimes accompanied with brown scattered blotches (Reeve, 1968). Due to similar tissue discolorations occurred in the tubers, sometimes these discolorations might confuse and mislead differentiating the BH disorder. That happens due to the overlapping with other internal physiological disorders induced in potato tubers showing similar symptoms with BH, depended on the severity of the disorder indicated (i.e. BC, brown centre; internal necrosis; HH; hollow heart; etc.). It has been proposed that BC is the precursor for HH development and possibly the initial step for BH induction (Reeve, 1968). Hooker (1981) proposed that BH may arise from internal heat necrosis or internal brown mahogany tissue discoloration. These statements are misleading as almost all internal physiological disorders induced in potato tubers share a common feature; that of brown tissue discoloration and cell necrosis in some cases (Dinkel, 1963; Van Denburgh et al., 1980, 1986; Bussan, 2007; Sowokinos, 2007; Wale et al., 2008). Craft *et al.* (1958) reported brown tissue discolorations in the central tuber tissues developed due to the low temperature injury in potatoes cvs. Russet Rural and Kennebec packed in ventilated polyethylene bags stored at 0^oC for ca. 4 months. Low temperature injury in potato tubers may be caused after storage at those cold temperatures slightly above the freezing point (0-2.5^oC), but the disorder may also be induced at 3.8-4.4^oC dependent on the cultivar. Brown tissue discoloration initially occurs

in vascular ring and the outer part of the tuber, but severe damage may result in grey and brown to black discoloration symptoms in the internal tuber tissue (UNECE 4th session note, 2011). However, its symptoms may overlap with other physiological disorders and pathogen diseases (Smith, 1978; Hooker, 1981; Wale *et al.*, 2008). Also, tissue discolorations in the central tuber part observed from Craft *et al.* (1958) were similar to those of BH symptoms (dark internal discoloration in the central tuber part) and not symptoms of low temperature injury. It is obvious that cold initial storage temperature may influence the incidence of BH-like symptoms or symptoms of similar internal physiological disorders. Nevertheless, it is still unclear whether brown tissue discolorations indicated herein were stimulated or induced at very low initial storage temperature and then exacerbated during shelf-life evaluation at either 15 or 20^{o}C. The temperature and exposure period in which BH shows greater incidence could not readily be predicted. That said, because in both years tissue discolorations were indicated randomly between the sampling days over storage time and yet BH incidence was equally evident in both storage conditions (air or under various gas combinations). It may be assumed that BH or BH-like symptoms may slowly develop at low temperatures without O_2 exclusion in the external atmosphere. On the other hand, the fact that BH can develop in about 100% of the tubers at very high temperatures (> 35-44^{o}C) may not be linked with shelf-life conditions, unless this happens when tubers are transported as mentioned previously.

Furthermore, it has been suggested that physiological disorders and tissue discolorations may be initiated during pre-harvest conditions such as growth conditions, soil temperatures and soil types, water logging (flooding), calcium deficiency (Link *et al.*, 1932; O'Brien and Rich, 1976; Bussan, 2007; Wale *et al.*, 2008; Palta, 2010; Zommic *et al.*, 2013). It was previously stated that cool growing conditions at 10-15^{o}C soil temperatures BC may be expressed in growing potatoes cv. Russet Burbank (Van Denburgh *et al.*, 1980, 1986). Zommic *et al.* (2013) reported that warm soil temperatures during potato bulking resulted in

severe BH incidence 270 days after harvest after storage at 9^{o}C. Since growing conditions affect the potato tuber yield some pre-harvest practices to control and avoid/reduce BH have been proposed recommending the use of well-drained fields with good soil porosity through tillage and organic matter to avoid excessive irrigation (Robinson and Secor, NDSU; Wale *et al.*, 2008). Light sandy soils should be avoided and under sunny and hot weather conditions at harvest period potato tubers have to be removed from hot soils when vines are dead (O'Brien and Rich, 1976; Smith *et al.*, 1978). Moreover, during storage good ventilation must be provided and storage temperature should be controlled.

8.1.1. Effect of tuber size and weight on the incidence of BH

The tuber size (namely length and maximum equatorial diameter) and tuber weight were also examined as additional factors influencing the incidence of BH. In year 1 (2011-2012), potato tubers from all three stocks analysed were about same in size (ca. 102 mm in length, 70 mm in diameter) and weight (ca. 240 g). Stock 23 (susceptible to BH) showed ca. 3-times greater BH incidence compared to stock 20 (susceptible to BH); generally, none of those dimensions measured nor did the weight have any effect on BH incidence. Also, in year 2 (2012-2013) all tubers derived from all three potato stocks and used in both experiments were similar in weight (ca. 241-272 g) and size (ca. 91–103 mm in length and 73-76 mm in diameter). Thus, it cannot be concluded that tuber weight and size have an effect on the incidence of BH due to the low indication of tissue discoloration. Once those brown tissue discolorations are initiated in the central part of the tuber (pith), it could be hypothesized that the narrowness of the pith which is positively associated with the tuber length as Tai and Misener (1994) pointed out and combined with the osmoregulation might have a possible role in tissue discoloration. However, longer tubers have narrow pith and pith cells localized in various tuber parts (namely central, stem and bud end) may vary in size (Reeve *et al.*, 1971;

Mogen and Nelson, 1986). From those findings it cannot truly be said that heavier and bigger sized tubers have greater incidence to brown tissue discoloration (Stewart and Mix, 1917). It has been reported that brown tissue discolorations may be developed in newly formed tubers as well (Van Denburgh *et al.*, 1980, 1986; Bussan, 2007; Zotarelli *et al.*, 2012) and therefore BH symptoms are more evident in medium sized tubers with ca. 60-70 mm in diameter (unpublished survey).

8.2. Effect of storage temperature and shelf-life conditions on the respiration rate of potato stocks with different susceptibility to BH

From harvest to consumption potatoes may undergo several physiological and compositional changes. In between, post-harvest reflects those techniques used to maintain the commodities quality providing fresh and healthy products to the markets and subsequently to the consumers (Khanal and Uprety, 2014). Generally, storage temperature is the most important factor controlling the postharvest life of stored commodities and thus affecting the metabolic processes such as respiration rate. Fresh potatoes are commercially stored at very low temperatures (3-4^{o}C) in order to extend their storage life by lowering their respiration rate providing year availability and marketability, thus creating desirable conditions for better shelf-life storage (Gast, 1991; Kleinkopf, 1995; Mathooko, 1996; Thompson, 1996; Nourian *et al.*, 2003; Zommick *et al.*, 2014). Respiration rate is considered key factor of stored potato tubers and may be used as a monitoring tool of their physiological activity during storage life as increases in respiration rate may reflect changes of the physiological status (Fennir *et al.*, 2003). It is well known that higher temperatures effectively increase the rate of respiration as very cold storage temperatures (< 5^{o}C) do as well (Dwelle ans Stallknecht, 1978; Workman *et al.*, 1979; Duplessis *et al.*, 1996; Zhou and

Solomos, 1998; Kumar, 2011). Under optimal storage temperatures when O_2 is efficiently supplied BH disorder is not developed, but may be induced at extreme high or low temperatures where higher O_2 levels are demanded. Also, BH development may also occur at around 5^{o}C but longer time is required (Bartholomew, 1916; Davis, 1928; Link *et al.*, 1932; O'Brien and Rich, 1976; Smith, 1978; Hooker, 1981; Wale *et al.*, 2008; Kumar Chaurasia, 2009; Voss, UC Davis; Robinson and Secor, NDSU). It is believed that very low storage temperature may cause sub-oxidation in potato tubers leading to tissue discoloration and yet BH development. On the other hand, increased CO_2 levels during storage may result in greater membrane permeability, physiological disorder development and tissue discoloration (Hooker, 1981).

In this project, an attempt to mimic the shelf life conditions of potato cv. Maris Piper stocks with different susceptibility to BH was conducted by initially storing the potato tubers at very low storage temperature (1.5 and/or 3^{o}C) and then transferring them at a higher storage temperature for shelf life evaluation. Respiration rate measurements were carried out at 15^{o}C in year 1 and 20^{o}C in year 2. In year 1 study, a similar pattern distinguishing significant differences in respiration rate between potato stock susceptibility to BH was not clearly shown over storage time except during baseline measurements. There were some significant differences in CO_2 production between the stocks analysed but were random among the sampling days over storage time. Similarly in year 2, even though there were some significant differences in respiration rate between those stocks analysed in both experiments; however, a relation with BH incidence was not observed. That might happen due to low BH incidence indicated during this project or because respiration rate was recorded in a jar containing both affected and non-affected tubers in the case of the susceptible stocks and also one or three tubers were placed in the jar (see Chapter 3, session 3.4). Furthermore, according to the findings in both years it seems that differences in respiration rate were due to low

initial storage temperature. It could also be said that O_2 was not really depleted during storage in year 1 (in 21% O_2 or 18-19% O_2 and 10% CO_2) and during the 1st experiment of year 2 (in 21% O_2), thus BH incidence was low; however, when potato tubers stored at various gas combinations (*viz.* A: 21% O_2, B: 10% CO_2, C: 10% O_2 and D: 5% O_2) it was shown that respiration rate was lower at 5% O_2 as it was expected, but still BH did not show greater incidence (Lipton, 1967).

Furthermore, increased respiration rate after storage at 10% CO_2 with 18-19% O_2 was observed in both years it was due to CO_2 absorption and the CO_2 rate results were in line with those reported by Perez-Trejo *et al.*, (1981). In fact, in year 2 analysis respiration rate results revealed that the non-susceptible to BH stock (stock 3) showed greater CO_2 production when held under 10% CO_2 storage compared to both susceptible stocks (7 and 12). It was worth noting that in year 1, a similar trend in respiration rate was followed by all stocks analysed which was higher respiration rate for those tubers initially stored at 1.5 compared to 3^{o}C. That trend was obvious in both storage conditions but more evident under CA storage (10% CO_2). It is well known that cold temperatures (1-4^{o}C) will stimulate the respiration rate (Craft, 1963; Workman, et al., 1979; Zhou and Solomos, 1998), but it seems that when temperature is further decreased nearly to those chilling temperatures (0^{o}C) respiration rate is higher and that is probably correlated with the sugar content of potatoes (Khanal and Uprety, 2014).

Even though the incidence of BH was not great under those storage conditions studied in this project, it could still be hypothesized that under very low or extreme high temperatures respiration rate will be increased and potato tubers will possibly experience a sub-oxidation situation. If tubers start respiring faster, then O_2 will not be able to reach the central part of the tuber and greater amount of CO_2 will be formed and trapped within the tissue resulting in cellular impairment, necrosis and tissue discoloration (Bartholomew, 1916; Hooker, 1981;

Kumar Chaurasia, 2009). However, respiration rate as a gas exchange process is always associated with those external atmospheric conditions surrounding the potato tubers, the internal gas concentrations and the gas diffusivity within the tuber tissues.

8.2.1. Gas diffusivity of potato tubers and the role of packaging

In potato tubers, O_2 diffusion initially occurs through the lenticels of the skin that are the dominant barriers, then passes through the flesh to the intercellular spaces where eventually the respiration takes place in the cytoplasm-mitochondria, while CO_2 is released following the opposite path (Wigginton, 1973; Banks and Kays, 1988; Weber, 1990; Geigenberger *et al.*, 2000; Ho *et al*., 2010). Potato tubers are compact crops having low porosity with only 1-2% of intercellular space volume in the tissue (Banks and Kays, 1988; Scotsmans *et al.*, 2003; Ho *et al.*, 2006). As gas-filled intercellular spaces are believed to pose the main pathway for gas diffusion (Devaux, 1891; Woolley, 1962; Weber, 1990), x-ray micro-computed tomography was used in year 3 (2013-2014). Once BH is associated with O_2 depletion in the central tuber part (heart), aim of this study was to visualize the tissue structure in flesh and heart tuber tissue of potato cv. Maris Piper stocks with different susceptibility to BH [stock 10 (susceptible to BH) and stock 4 (non-susceptible to BH)]. Both micro-CT scans and 3D images showed that the gas-filled intercellular spaces of flesh and heart tissue were not well connected and varied in size and shape. This might suggest that due to differences in cell structure, different intercellular space connectivity may be formed influencing further large gradient of diffusion the gas diffusion within a tissue. Therefore, the gas-filled intercellular spaces might partially be filled up with water or sap posing an additional resistance to gas diffusion as the cell walls becoming hydrated. It is also believed that CO_2 diffusion has greater solubility in water and transports faster in the liquid phase

while O_2 mainly diffuses through the gas phase (Woolley, 1962; Himmelblau *et al.*, 1965; Abdul-Baki and Solomos, 1994; Ho *et al.*, 2006a, 2007, 2011; Licausi and Perata, 2009).

It has been reported that adequate O_2 concentration is contained in the intercellular spaces of the tuber tissue due to low respiration rate of the tuber under regular storage conditions (Burton, 1950; Woolley, 1962; Wigginton, 1973; Abdul-Baki and Solomos, 1994); however, partial hypoxic and anoxic atmospheres might occur at the centre of the tuber under adverse environments resulting in physiological changes and tissue cell impairment and yet cell death (Geigenberger *et al.*, 2000; Ferreira de Souza *et al*, 2002; Verboven *et al.*, 2008; Zabalza *et al.*, 2009). When O_2 concentrations decline within a tissue that is probably because the external supply of O_2 is insufficient or needed in order the gas to be driven into the tissue (Ferreira de Souza *et al.*, 2002; Geigenberger *et. al.*, 2003). It is believed that plant tissues have O_2 sensing systems and develop adaptive responses in order to reduce O_2 consumption and to keep their metabolic energy. That said, as some tissues may have the ability to develop pre-adaptation mechanisms to O_2 falling levels and might delay or tolerate anoxia later. However, those adaptive responses require decrease in respiration rate and yet lower adenine triphosphate (ATP) consumption. Thus, changes in metabolic processes occur and cellular energy may be provided by fermentation pathways which in turn may lead to cell impairment (Geigenberger *et al.*, 2000). It is suggested that tuber tissues may be turned hypoxic or anoxic even at normal air conditioning (21% O_2). Yet, when tissues are experiencing hypoxia or anoxia, re-entry of oxygen may cause an imbalance in the cellular redox resulting in reactive oxygen species (ROS) accumulation due to oxidative stress (Geigenberger *et al.*, 2000). That may also happen under low storage temperature due to a cold-induced stress (Cheynier *et al.*, 2009). ROS are produced during aerobic respiration, but plant tissues have the ability to keep the redox status in balance using scavenging systems including enzymatic and non-enzymatic antioxidants (i.e. phenolic compounds) that detoxify

ROS (Hodges, 2003; Sen, 2012). Furthermore, it has been reported that wound-induced enzymes like PAL which is the precursor for the phenylpropanoid and flavonoid pathway may be increased due to cold stress (Cheynier et al., 2009); however, at very low oxygen levels its activity is inhibited (Zhou and Solomos, 1998; Geigenberger *et al.*, 2003). In this project, the gaseous differences in which BH is induced could not be predicted. Furthermore, it has been reported that when O_2 falls below 5% then BH might be developed and that is more evident when tubers are packed (Lipton, 1967; Hooker, 1981; Adrian Briddon, personal report, 2011). This suggests that BH is initiated under hypoxic or anoxic environments and might be developed due to oxidative stress caused. Herein, it seems that the initial cold storage temperature (1.5^{o}C) was the main factor affecting both respiration rate and incidence of BH-like symptoms even though a relation between them was not observed. It could also be assumed that at low storage temperatures BH or BH-like disorders may be developed slower when O_2 is not really depleted externally. Also phenolic related compounds were mainly accumulated in the control heart part of the tubers derived from susceptible to BH stocks and it is believed that was a response to cold-induced stress. It was also observed that tuber tissues derived from non-susceptible to BH stocks did not show indications of oxidative stress damage. As for the discoloured samples, indications of cell membrane damage and lipid peroxidation were evident. That might happen due to ROS accumulation caused by oxidative stress. However, differences between susceptible and non-susceptible to BH stocks might be explained due to different adaptive responses under cold storage and yet different gene expression and regulation in this project. According to Geigenberger *et al.* (2003) there was a concern about the previous studies where researchers were mainly focusing on the low storage temperature effect on tissue metabolism underestimating probably that growing tubers may undergo hypoxia at ambient growth conditions.

Also, it is believed that packaging and different film types will further affect the BH development. The packaging material might be a crucial factor affecting the gas exchange rate which is controlled by the number and dimensions of the perforations of the material. It may be expected that O_2 and CO_2 exchange is dependent on the temperature and the atmospheric pressure (Fonseca *et al.*, 2000; Lange, 2000). Potatoes are mainly packed washed in bags containing 1, 2.5, 5 or 10 kg of tubers. In the UK, low-density polythene (LPDE) film with small holes for some ventilation is preferred in the markets. Netted bags that provide greater ventilation may also be used. However, there are many factors selecting the suitable packaging material including the manufacturing of the film, the cost and yet the consumer issues (Lange, 2000). After packaging trials on 15 Maris Piper stocks, A. Briddon reported that BH incidence was 2-fold higher in punched polythene packed potatoes compared to those unpacked after storage at 20^{o}C for 13 days. He also concluded that initial storage temperature at 1.5^{o}C effectively increased BH incidence mainly in packed potatoes compared to 3.5^{o}C, but BH symptoms were accompanied with grey diffusion (suggested as total % of BH symptoms) were more evident in unpacked tubers (Adrian Briddon, personal report, 2011). Later, similar packaging trials on the same Maris Piper stocks used in year 1 [stock 20 (susceptible to BH), stock 23 (susceptible to BH and stock 12 (non-susceptible to BH) were conducted by A. Briddon using two film types (punched hole and laser perforated). It was reported that BH incidence was greater in those tubers packed with laser perforated film compared to those packed in punched polythene material. (Adrian Briddon, personal report, 2011). Furthermore, previous storage at 1.5°C, irrespective of film type, and packaging stock 23 in laser perforated film after storage at 3.5°C resulted in relatively high incidences of BH and total BH. Yet, stock 12 which was considered as non-susceptible to BH also showed BH incidence. In this project, a similar trend in BH-like symptoms or total BH symptoms was not observed in unpacked potato tubers, but BH incidence was also greater

when tubers had initially been stored at 1.5^{o}C. Also, stock 12 (non-susceptible to BH) showed no evidence of BH-like symptoms under those storage conditions used in this project. From those findings, it is still unclear how potato stock susceptibility might be determined and there is a confusion of what are the real symptoms that govern the BH disorder.

8.3. Biochemical changes in potato stocks with different susceptibility to BH as influenced by storage temperature and shelf-life conditions

8.3.1. Effect of storage temperature and shelf-life conditions on the sugar content of potato stocks with different susceptibility to BH

Sugar content in potato tubers stored at very cold storage temperatures may be a good indicator of their compositional changes during the storage life (Kumar, 2011). In this project, the major sugars present in potato tubers namely fructose, glucose and sucrose were quantified in those tubers that were initially stored at 1.5^{o}C where the incidence of BH was greater. Sucrose, the substrate for fructose and glucose biosynthesis, may either be catalysed by sucrose synthase or invertase enzymes. At cold storage temperatures, inactivation of invertase inhibitor leads to expression of invertase resulting in rapidly sucrose degradation to reducing sugar accumulation (Zhou and Solomos, 1998; Bologa *et al.*, 2003; Kumar, 2011). This cold-induced phenomenon known as 'low temperature sweetening' (TLS) or 'cold-induced sweetening' (CIS) has been extensively studied and to date is still of great interest (Muiller-Thurg, 1882; Pressey, 1969; Sowokinos, 2001; Malone *et al.*, 2006; Kaul *et al.*, 2010; Janksy *et al.*, 2014; Zhang *et al.*, 2014). In year 1 (2011-2012) reducing sugars (fructose and glucose) were initiated and rapidly accumulated after baseline where tuber subsamples were stored at 1.5^{o}C. In general, fructose and glucose were ca. 2 times higher in

heart than in flesh tissue of stock 20 (susceptible to BH) and stock 12 (non-susceptible to BH) with no really significant differences between those stocks. On the other hand, the cold temperature effect on sugar content was more evident in both control and discoloured flesh and heart tissue samples of stock 23 (susceptible to BH) from 8 to 20 weeks of storage. Generally in year 2, reducing sugar content was also ca. 2-fold higher in heart than flesh tissue samples of both stocks when measured at 20^{o}C. Reducing sugar content of stock 7 (susceptible to BH) was similar to that of stock 23 (susceptible to BH) measured in year 1. However, in year 2 fructose and glucose were not really increased during storage time. Also, changes in sucrose content of stock 3 (non-susceptible to BH) were not shown over storage time, but it was significantly lower compared to stock 7.

To date, no relation in reducing sugar accumulation and BH disorder has ever been reported. Increased sugar content in potato tubers is related with brown to black discoloration; however, that is caused upon frying via Maillard reactions (Shallenberger *et al.*, 1958; McCay *et al.*, 1990; Cottrell *et al.*, 1993; Stark *et al.*, 2003; McKenzie *et al.*, 2005; Zommick *et al.*, 2013). However, it has previously been pointed out that tuber tissues can be affected by other internal physiological disorders in potatoes such as brown centre, hollow heart and internal brown spot may accumulate reducing sugars (Davies, 1998; Bussan, 2007). In year1, the incidence of BH was greater compared to year 2. Brown tissue discolorations were mostly indicated in heart tissue samples of both susceptible to BH stocks (stock 20 and stock 23), but a few heart samples of stock 23 showed more intense brown to black tissue discoloration. It was observed that stock 23 which showed greater tissue discoloration (ca. 3-fold compared to stock 20) had also higher reducing sugar content compared with stock 20 and stock 12 (non-susceptible to BH). That high sugar accumulation in stock 23 was observed in both tissue samples suggesting that the whole tuber was affected after storage at low cold temperature. Sugar content may vary from cultivar to cultivar but it has been shown

that it might be more accumulated in the pith indicating that its mobilization is more active towards this tuber area (Baijal and Van Vliet, 1966; Weaver *et al.*, 1978). A study on the reducing sugar content in different tuber parts of six potato cultivars by Weaver *et al.* (1978) showed that sugar content variation between those cultivars after storage at 7^{o}C for 2-4 months and/or after reconditioning at 20^{o}C for 3 weeks; however, it was shown that cvs. Kennebec and White Rose had higher reducing sugar content in the core tuber part while Russet Burbank the lower. Dwelle and Stallknecht (1978) also reported slightly higher total and reducing sugar content in central pith tissue samples of Kennebec compared to Russet Burbank after storage at 1.7^{o}C. Those three potato cultivars seem to have different susceptibility to physiological disorders. O'Brien and Rich (1976) suggested that Russet Burbank cultivar is resistant to BH while according to Robinson and Secor (NDSU, 2014) this cultivar is susceptible to internal heat necrosis (IHN) and brown centre (BC) as Van Denburgh *et al.* (1980, 1986) has previously pointed out. Kennebec cultivar was found being susceptible to low temperature injury and BH (Craft *et al.*, 1958; Butchbaker *et al.*, 1967) Also, Lipton (1967) reported BH incidence in White Rose potato tubers after storage in 0.5-1% O_2 at 15 – 20^{o}C with ca. 2-fold lower glucose concentration in the outer and inner parts of the tubers compared to those held in air (21% O_2). Furthermore, Zhou and Solomos (1998) showed increase in sugar content of Russet Burbank potatoes in air at 1^{o}C, but strong inhibition after storage in 1.5% O_2 at 1^{o}C due to hypoxia. However, in this project sugars were much higher compared to those results previously published, but it seems that different storage conditions and storage temperature affect the sugar accumulation.

Moreover, storage at cold temperatures may lead to cellular stress response by negatively affecting the membrane lipid composition and subsequently leading to changes in fatty acid degradation, ion leakage thus greater membrane permeability and cell impairment are caused (Sowokinos *et al.*, 1985, 2001; Wills, 1989; Berkel *et al.*, 1994; Davies, 1998;

Wismer *et al.*, 1998; Blenkishop *et al.*, 2004; Kumar, 2011). Shekhar *et al.* (1979) stated that membrane permeability is greater for tubers stored at cold temperatures. Thus, potato stocks with different susceptibility to BH and similar physiological disorders may also show susceptibility to lipid peroxidation (Davies, 1998) as it has been reported that some potato cultivars with higher unsaturated fatty acid content have lower sugar accumulation (Spychalla and Desborough, 1990). From those findings, it could be said that reducing sugar may be increased due to brown tissue discolorations and be related with BC and similar disorders. BH and BH-like disorders are initiated in the very central part of the tuber the pith area. It could also be hypothesized that pith (which is characterized as the watery and translucent area of the tuber) becomes even translucent at cold-storage temperatures, thus a greater sugar accumulation is observed. It has been suggested that small translucent, watery spots are prior signs of BH development in pineapple (Zhou *et al.*, 2003). Although, it is likely that tuber tissues showing more less or more intense brown tissue discoloration may contain higher sugar content as it was observed for both tuber tissue samples of stock 23 (susceptible to BH) in year 1; however, the very low indication of dark brown to black symptoms and the fact that reducing sugar accumulation was similar between discoloured and control samples of stock 7 (susceptible to BH) in year 2 that cannot really highlight a relation with BH in this project.

8.3.2. Effect of storage temperature and shelf-life conditions on the phenolic content of potato stocks with different susceptibility to BH

Potato stocks with different susceptibility to BH could well be grouped according to their phenolic content and that was shown in both years (1 and 2). It should be noted that in year 1, phenolic content quantification was carried out in flesh and heart samples according to potato susceptibility to BH and both control and discoloured samples were merged and averaged in the case of both susceptible to BH stocks (20 and 23) due to low BH incidence

indicated. In contrast, in year 2 analysis phenolics were quantified in both control and discoloured tissue samples due to statistical balance needed. Phenylalanine and other aromatic acids as tyrosine and tryptophan are synthesized from the shikimate pathway (Herrmann, 1955; Dewick, 2002; Vogt, 2010; Payyavula *et al.*, 2012). In year 1, phenylalanine was more expressed in those heart samples of stock 23 (susceptible to BH) and that was more evident after 16 and 20 weeks of storage where stock 23 showed greater BH incidence. Yet, according to the PCA biplots, tryptophan was mainly accumulated in those discoloured heart samples of stock 23 after 20 weeks of storage. A similar trend in tyrosine content was followed by those heart samples of stock 23 (susceptible to BH) after 16 and 20 weeks where BH incidence was greater as well. On the other hand, in year 2 analysis phenylalanine varied in content between stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) and similarly for tryptophan a distinct trend was not shown, but heart samples of stock 7 showing light brown discoloration (BCL) tended to accumulate more tryptophan. From those findings, a full conclusion on the amino acid accumulation cannot be made. Yao *et al.* (2005) reported that both phenylalanine and tyrosine amino acids may positively be activated by tryptophan.

Deamination of phenylalanine via the enzyme phenylalanine ammonia lyase (PAL) leads to phenylpropanoid pathway (Joos and Halbrock, 1992; Gerasimova *et al.*, 2005) by generating a large amount of secondary metabolites including phenolic compounds such as hydroxycinnamic acids and flavonoids. In this project, phenolic content varied between year 1 and year 2 analyses; however, it was shown that chlorogenic acid and its isomers namely neo- and crypto-chlorogenic acid were the most important variables differentiating potato stocks with different susceptibility to BH. It has been reported that chlorogenic acid is more accumulated in the outer tuber parts than the inner with the peel and cortex accumulating the highest content (Craft *et al.*, 1958; Zucker and Levy, 1958; Dao and Friedman, 1992;

Friedman, 1997). However, in this project it was shown that chlorogenic acid tended to be more accumulated in the heart tissue samples of stocks susceptible to BH. Particularly, in year 1 chlorogenic acid tended to be more accumulated in heart tissue samples of stock 23 which showed greater BH incidence during the 1st year experiment and yet highly concentrated in control heart samples of stock 23 over storage time. A similar trend was shown in year 2, where chlorogenic acid content was more accumulated in the heart samples (both discoloured and control) of that stock 7 (susceptible to BH). On the other hand, its content was much higher in year 2 as samples of stock 7 (susceptible to BH) contained ca. 2-9 times higher chlorogenic acid compared to stock 23 (susceptible to BH) in year 1. It should be noted that all stocks analysed in both years have been grown at different locations and growing conditions and seasons, so that might have an impact on PAL enzyme activity (Hamouz *et al.*, 2010; Ieri *et al.*, 2011; Adamo *et al.*, 2012; Payyavula *et al.*, 2012). Furthermore, a similar trend in chlorogenic acid isomers accumulation was observed in both years. Neo-and crypto-chlorogenic acids tended to be more accumulated in the flesh tissue samples of non-susceptible to BH stocks. Yet, crypto-chlorogenic acid showed greater accumulation in the heart samples of the susceptible to BH stocks. Similar concentrations of both isomers have previously been reported in white fleshed potato cultivars (Lachman and Hamouz, 2005; Navarre *et al.*, 2011; Payyavula *et al.*, 2013). The fact that chlorogenic acid showed not difference in concentration in those flesh samples might be explained due to no discoloration indicated. Im *et al.* (2008) reported that chlorogenic acid was lower in the pith tuber area than in the outer. Thus, it could be said that chlorogenic acid was more accumulated in those heart samples due to cold stress response.

Furthermore, it is well documented that due to low storage temperature a cold-induced PAL enzyme triggers the biosynthesis of phenolic compounds that accumulate in response to stress (Cheynier *et al.* 2009). Cheynier *et al.* 2009 has pointed out that low temperature effect

should trigger both PAL activity and those enzymes involved in the phenylpropanoid and flavonoid pathway in order for the phenolic compounds to be accumulated. In year 2, increased accumulation of flavonols such as rutin and quercetin-3,4-O-diglucoside in those samples of the non-susceptible stock 3 was observed. This might explain differences in phenylalanine gene expression between susceptible and non-susceptible to BH stocks under storage at cold temperatures. Low storage temperature also triggers the accumulation of reactive oxygen species (ROS) that negatively affect the cellular membrane integrity. Plants have the ability to react against those ROS developing membrane repair mechanisms, but this ability depends on many factors. It is well known that phenolic compounds have scavenging activities along with vitamin C against reactive oxygen species (ROS) (Brown *et al.*, 2005; Takahama *et al.*, 2002). Also, it has been reported that vitamin C in potatoes decreases at low storage temperature (Kawakami *et al.*, 2000; Dale *et al.*, 2003) and that scavenging system may be also lowered, thus leading to greater cell membrane degradation and lipid peroxidation. Further, cell membrane stability may negatively be affected by calcium deficiency (Palta, 2010). Although vitamin C and scavenging system properties were not examined in this present study, it may be hypothesized that non-affected tuber tissues may be adapted differently under low storage temperature (Purvis and Shewfelt, 1993) and also have greater scavenging activity.

In terms of tissue discoloration, generally less and more intense brown tissue discolorations were indicated in the heart tissue samples of those susceptible to BH stocks in both years and classified as (*viz.* BH, dark brown to black; BC, brown centre; BCL, brown centre light and pith). In year 1, stock 23 (susceptible to BH) showed ca. 3-times higher BH incidence compared to stock 20 (susceptible) and stock 12 (non-susceptible) and that was more apparent after 16 and 20 weeks of storage. According to the results, after 16 and 20 weeks most of the phenolic compounds and reducing sugars quantified were mostly

accumulated in those heart samples of stock 23. Furthermore, a similar trend was followed in year 2. That was higher accumulation of sugar, chlorogenic acid and crypto-chlorogenic acid in those heart samples of stock 7 (susceptible to BH). The biochemistry of the BH disorder is scarce. Initially, Bartholomew (1916) reported that tyrosinase is activated at high temperatures (< 38^{o}C) thus tyrosine is being oxidized resulting in black tissue discoloration. However, according to Smith *et al.* (1942) no changes in tyrosine content were observed after storage at 40^{o}C. Later, Reeve (1968) after histochemical studies on affected tuber tissues showing BH symptoms concluded that both chlorogenic acid and tyrosine were present. It is well known that both chlorogenic acid and tyrosine are the major phenolic compounds present in potato tubers with tyrosine being more accumulated in the inner part of the tuber (Craft *et al.*, 1958; Reeve, 1968) and both compounds are adequate substrates for enzymatic oxidation; tyrosine by polyphenol oxidase (PPO or tyrosinase) and chlorogenic acid by PPO or peroxidase (POD) (Takahama *et al.*, 2004; Adams, 2007). For instance, Zhou *et al.* (2003) reported that PPO is the responsible enzyme for tissue discoloration caused by BH in pineapple fruit and BH development is due to the *de novo* synthesis of PPO rather a pre-existing one concluding that there are also many other factors triggering PPO activity. Also, black discoloration is mainly caused by tyrosine oxidation while chlorogenic acid oxidation results in brown pigments (Adams *et al.*, 2007). However, there is confusion between substrates and enzyme activity related to tissue discoloration and the final colour of the oxidation products (Werij *et al.*, 2007). Generally, approaches on enzymatic brown discolorations are carried out either focussing on the substrate or the enzyme where ideally both factors should be examined. However, it has been shown that PPO may be the main enzyme involved in enzymatic browning in potato tubers (Vaughn *et al.*, 1988). Furthermore, it has been reported that silencing the PPO gene in transgenic potato lines a reduction in enzymatic discoloration was observed (Bachem *et al.*, 1994; Shepherd, *et al.*, 2014). Herein,

chlorogenic content was much higher than that of tyrosine, thus ideally it could be hypothesized that the former compound has greater possibility to be involved in tissue discoloration. However, it was more accumulated in those control heart samples of stock 23 (susceptible to BH) in year 1 and both discoloured and control samples of stock 7 (susceptible to BH in year 2. Friedman (1997) showed no correlation between chlorogenic acid and discoloration. According to Corsini *et al.* (1992) higher accumulation of tyrosine and free amino acids were related with higher tissue discoloration. In contrast, Mondy and Munshi (1993) reported that even though higher levels of free tyrosine were positively correlated with discoloration, tyrosine amount did not seem to be the factor for determining enzymatic discoloration. It is still unclear if tyrosine or chlorogenic acid or other phenolic compounds that were not quantified contributed to those brown tissue discolorations indicated in this project and it is more likely that caused due to initial storage temperature as response to cold-induced stress as BH incidence was observed in both storage conditions and yet was greater in those tubers stored in air than in various gas combinations.

8.3.3. Untargeted metabolomics approach on selected samples of potato stocks with different susceptibility to BH

Untargeted metabolomics analysis is being used in order to detect and identify the total content of a sample providing information on which metabolites are the most highly concentrated (de Voss *et al.*, 2007; Patti *et al.*, 2012). In this project, selected discoloured and control samples derived from potato stocks with different susceptibility to BH were used to identify as many as possible metabolites that might have a possible link with the BH development. The purpose of this untargeted metabolomic approach was to find metabolic differences between discoloured and control samples derived from a susceptible stock and

moreover, to identify metabolic differences in potato stock susceptibility to BH comparing control samples derived from one susceptible and one non-susceptible to BH stock.

The incidence of BH was greater in year 1 (2011-2012) than in year 2 (2012-2013) and less and more intense tissue discoloration in the heart tissue were observed and classified as (*viz.* BH, dark brown to black; BC, brown centre; BCL, brown centre light and pith). That tissue discoloration classification was carried out in that way because it was assumed that less intense brown tissue discoloration may be the initial steps for the BH development. According to the 3D PCA scatter plots a good separation of samples having different tissue discolorations with intensity BH > BC > BCL > pith > control based on a coefficient of variation of 10% it was observed in both ion modes. However, a relationship between more intense (BH) and less intense tissue discolorations as initiators of BH could not be confirmed as the metabolite identification and regulation did not show a similar pattern in less intense brown discolorations (BC, BCL and pith) compared to control samples. That might have happened due to low sample replication. Generally, most of the known metabolites identified were fatty acids and also a plethora of unknown metabolites was observed. Few of the latter metabolites up regulations in discoloured tissue samples were showed. BH and BH-like symptoms in potato tubers may be easily distinguished due to the dark brown or black discoloration of the heart and flesh tuber tissue depended on the severity (Bartholomew, 1916; O'Brien and Rich, 1976; Wale *et al.*, 2008; Kumar Chaurasia, 2009). Thus a greater metabolite regulation was observed in those samples with BH-like symptoms as it was expected. Evidence of cell membrane lipid peroxidation and tissue damage was observed as unsaturated hydroxy fatty acids and lipid peroxidation products were identified. In addition, the fact that some of the unsaturated hydroxy fatty acids were present in control samples indicates that the cold storage temperature contributed to tissue cell membrane deterioration as all the samples analysed have been stored for more than 16 weeks at 1.5^{o}C. This

metabolite regulation might be related with response to stress and defence against ROS attack as result to cold storage temperature (Sen, 2012).

Metabolomic differences between control samples of potato stocks with different susceptibility to BH were more evident. In both years analysis, 3D PCA scatter plots showed a clear separation according to potato stock susceptibility to BH and significant regulations of known secondary metabolites such as glycoalkaloids, flavonoids and other phenylpropanoid related metabolites were observed. Flavonoids and steroidal glycoalkaloids both represent important groups of the secondary metabolism in plants and have extensively been studied in potatoes before (Harbone, 1959; Bostock *et al.*, 1982; Cantwell, 1996; Lewis *et al.*, 1998; Friedman *et al.*, 2006; Payyavula *et al.*, 2012, 2013).

Glycoalkaloids are considered as the toxic group of the secondary metabolites and are stimulated by many factors such as light exposure, temperature, storage conditions and mechanical injury (Jadhav *et al.*, 1980; Cantwell, 1996; Smith *et al.*, 1996; Simonovska and Vork, 2000). A-chaconine and a-solanine are the principal glycoalkaloids in potatoes accounting ca. 95% of the total glycoalkaloids, distributed in all parts of the potato plant and their content varies in the potato tuber [(peel > cortex > flesh – pith (not detectable)]. A-chaconine and a-solanine synergism results in toxic effects (Smith *et al.*, 1996; Friedman, 2006; Mandimika, 2008). In year 1, differences in glycoalkaloid content between control samples of stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) were shown. A down regulation of a-chaconine was observed in those samples of stock 23 regardless the tissue. On the other hand, solanine, solanidine and solasonine (another aglycone) were up regulated in flesh control samples of stock 12. Also, solanine and solanidine were up regulated in heart tissue samples of stock 12, but solasonine did not show any changes. In addition, no changes in glycoalkaloid content in heart control samples of stock 23 were observed. Jadhav *et al.* (1980) reported that total glycoalkaloid content was increased in

potatoes cvs. Russet Burbank, Norgold Russet and Pontiac with slight and severe BH and hollow heart (HH) incidence due to tissue damaged caused by BH and HH, but concluded that these were less potent factors stimulating the glycoalkaloid synthesis compared to other factors as light and mechanical injury. Increase in total glycoalkaloid content in potato cultivar Torrindon due to injury and tissue damage has previously been reported (Dale *et al.*, 1998). Glycoalkaloids are localized and accumulated in the vacuoles and the cytoplasm and may be transferred if the tissue is damaged (Väänänen, 2007). Herein, only control samples were analysed and yet no indications of glycoalkaloid accumulation in discoloured samples were observed. However, that glycoalkaloid accumulation might be an indicator of stress response (Lawley, 2013) as at maximum levels glycoalkaloid activity in potato cells might influence the membrane permeability causing disruption and leakage (Coria, *et al.* 2011).

Also, in year 2 analysis there were no significant regulations of glycoalkaloids between those control samples of Maris Piper stocks [stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH)]. All Maris Piper potato stocks in both years had initially been stored in air at 1.5^{o}C and analysed after storage at 15 and 20^{o}C in year1 and year 2, respectively. It has been reported that low storage temperature (0-5^{o}C) increases the glycoalkaloid content in potatoes due to stress caused (Dale *et al.*, 1998; Lawley, 2013). Also, it should be noted that all stocks of each year have grown in different locations and growing conditions. Thus, even though it has been proposed that Maris Piper cultivar is light-insensitive, different growing conditions and locations may affect the glycoalkaloid content in potatoes (Percival, 1999; Smith *et al.*, 1998; Sengun *et al.*, 2004, Khan *et al.*, 2013).

Flavonoids identification was observed in both years. Flavonoid pathway biosynthesis is initiated enzymatically by chalcone synthase catalysis and the pathway further proceeds with several enzymatic steps to other subclasses of flavonoids (Schijlen, 2007). It has been

reported that most of the flavonoids are present as glycosides synthesised by glycosylation namely a sugar attached to the aglyone using glycoyltransferases (Kim *et al.*, 2006, 2013; Aksamit-Stachurska *et al.*, 2008; Simkhada *et al.*, 2010). In year 1 analysis, two polyhydroxyflavones [(hibiscetin or 3,5,7,8-tetrahydroxy-2-(3,4,5-trihydroxy phenyl) chromen-4-one and 5,7,3',4',5'-pentahydroxy-3,6,8-trimethoxyflavone] showed an up regulation in those control samples of stock 23 (susceptible to BH) when compared with those control samples of stock 12 (non-susceptible to BH). In year 2, up regulation of quercetin-3-glucoside-7-rutinoside, quercetin-3-glucoside-7-rhamnoside (both diglycosides) and myricetin-3-rutinoside was observed in those flesh and heart samples of stock 3 (non-susceptible to BH) compared to flesh and heart samples of stock 7 (susceptible to BH). Similarly, quercetin-3-glucoside-7-rutinoside, myricetin-3-rutinoside, quercetin-3-rutinoside (or rutin) and two other flavonoids 3,5,7,2',5'-pentahydroxyflavone and 3,5,7,2',5'-pentahydroxyflavone + 5.129875 were all up regulated in stock 3 (non-susceptible to BH) regardless the tissue. Rutin (quercetin-3-rutinoside), myricetin-3-glucoside and similar flavonol glycosides and diglycosides identified in year 2 analysis have previously been reported in white and coloured potatoes (Lewis *et al.*, 1998; Navarre *et al.*, 2011; Payyavula *et al.*, 2012, 2013).

Furthermore, 4-oxoproline which is involved in proline metabolism showed down regulation in stock 23 (susceptible to BH) in year 1. It has been suggested that proline may be accumulated in plants as a physiological response against to biotic and abiotic stress and might influence the adaptive responses to the stressors and its accumulation may provide protection of cell function, membrane and enzyme activity (Cheynier *et al.*, 2009).

The identification of flavonoid compounds in both years and that of phenylalanine and quinic acid in year 1 analysis suggest differences in gene expression and regulation of the phenylpropanoid compounds and their biosynthetic pathway. A down regulation of the

known quinic acid which is a key metabolite for chlorogenic acid synthesis was observed in stock 23 (susceptible to BH). Phenylalanine which is the precursor for the phenylpropanoid and flavonoid pathway did not show any changes in flesh samples of stock 12 when compared with flesh samples of stock 23 and it was down regulated in heart samples of stock 12 compared to heart samples of stock 23. Targeted analysis of phenolic compounds showed that phenylalanine tended to be more accumulated in those heart samples of susceptible to BH stocks. Furthermore, rutin and quercetin-3,4-O-diglucoside showed greater accumulation in some flesh samples of stock 12 (non-susceptible to BH) in year 1 and in both tissues of stock 3 (non-susceptible to BH) in year 2. It has been reported that storage at cold temperatures triggers the PAL activity and yet those enzymes involved in the phenylpropanoid and flavonoid pathway in order the phenolic compounds to be accumulated (Cheynier *et al.*, 2009). This might explain differences in phenylalanine gene expression between susceptible and non-susceptible to BH stocks after storage at cold temperatures (1.5^{o}C) indicating different response and adaptation to a cold-induced stress. On the other hand, it is unknown whether the synthesis of those phenylpropanoid compounds was due to a pre-existing PAL or a de novo synthesis of the enzyme.

8.4. Conclusions

In conclusion, the cold initial temperature was the main factor influencing both respiration rate and compositional changes in potato tubers from stocks with different susceptibility to BH. However, a relation between respiration rate and BH could not be made. In general the incidence of BH was quite low proportionally to the tubers used in this project and yet greater in year 1 compared to year 2 studies. The fact that all Maris Piper potato stocks used in this project have grown at different locations and growing conditions explains differences in both physiological and biochemical changes observed and yet in BH incidence.

However, less and more intense brown discolorations were indicated in the heart part of those tubers derived from the susceptible to BH stocks in year 1 and year 2. It is obvious that cold initial storage temperature may influence the incidence of BH-like symptoms or symptoms of similar internal physiological disorders. Nevertheless, it is still unclear whether brown tissue discolorations indicated in this project were stimulated or induced at very low initial storage temperature and then exacerbated during shelf-life evaluation at either 15 or 20^{o}C. The temperature and exposure period in which BH shows greater incidence could not readily be predicted. That said, because in both years (1 and 2) tissue discolorations were indicated randomly between the sampling days over storage time and yet BH incidence was equally evident in both storage conditions (air or under various gas combinations). It may be assumed that BH or BH-like symptoms may slowly be developed at low temperatures without O_2 exclusion in the external atmosphere. Similarities in biochemical changes between susceptible and non-susceptible to BH stocks were observed in both years (1and 2). A similar trend in sugar content was observed in both year analyses where sugars tended to be more accumulated in the susceptible to BH stocks and that might be an indicator of stock susceptibility.

Also the fact that chlorogenic acid was highly accumulated in both control and discoloured samples of susceptible to BH stocks makes unclear whether is a marker for tissue discoloration and potato stock susceptibility to BH. Furthermore, the identification of glycoalkaloid and flavonoid compounds in control samples suggesting differences in gene expression and regulation under those storage conditions studied. However, it is still unclear whether these compounds may differentiate the potato stock susceptibility to BH. Further biochemical research is needed in order to confirm whether those findings are related with BH disorder. Also, it is still questionable of which are the real factors that govern the BH

disorder development and the symptoms that differentiating the disorder from similar internal physiological disorders induced in potato tubers.

CHAPTER NINE:

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APPENDICES

Appendix A)

1. Example of blackheart (BH) incidence of potato tubers in year 1 (2011-2012).

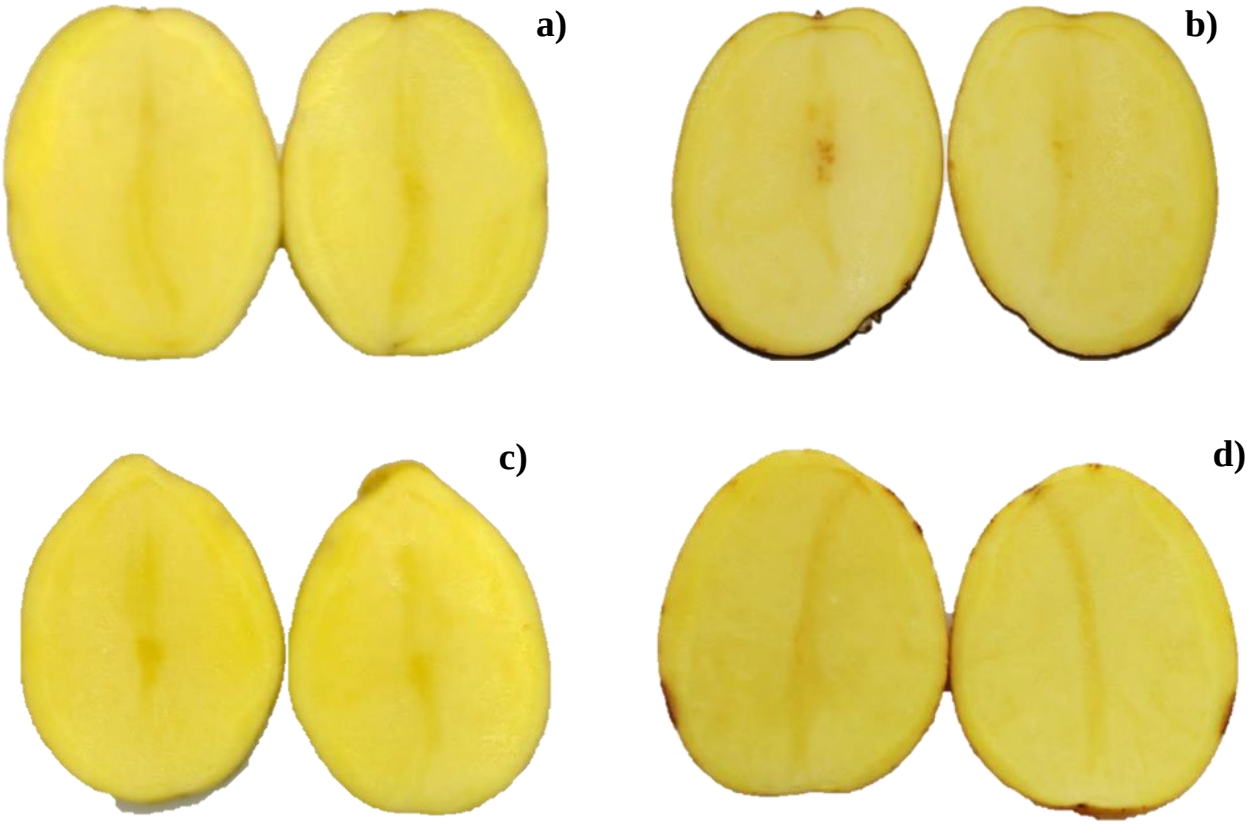

Figure 1.1 Example of BH incidence of potato tubers of stock 20 (susceptible to BH) after storage in air (a, b) or CA (c, d) at 15^{o}C. Tubers were initially stored at 1.5^{o}C (a, c) or 3^{o}C (b, d) for 8 weeks.

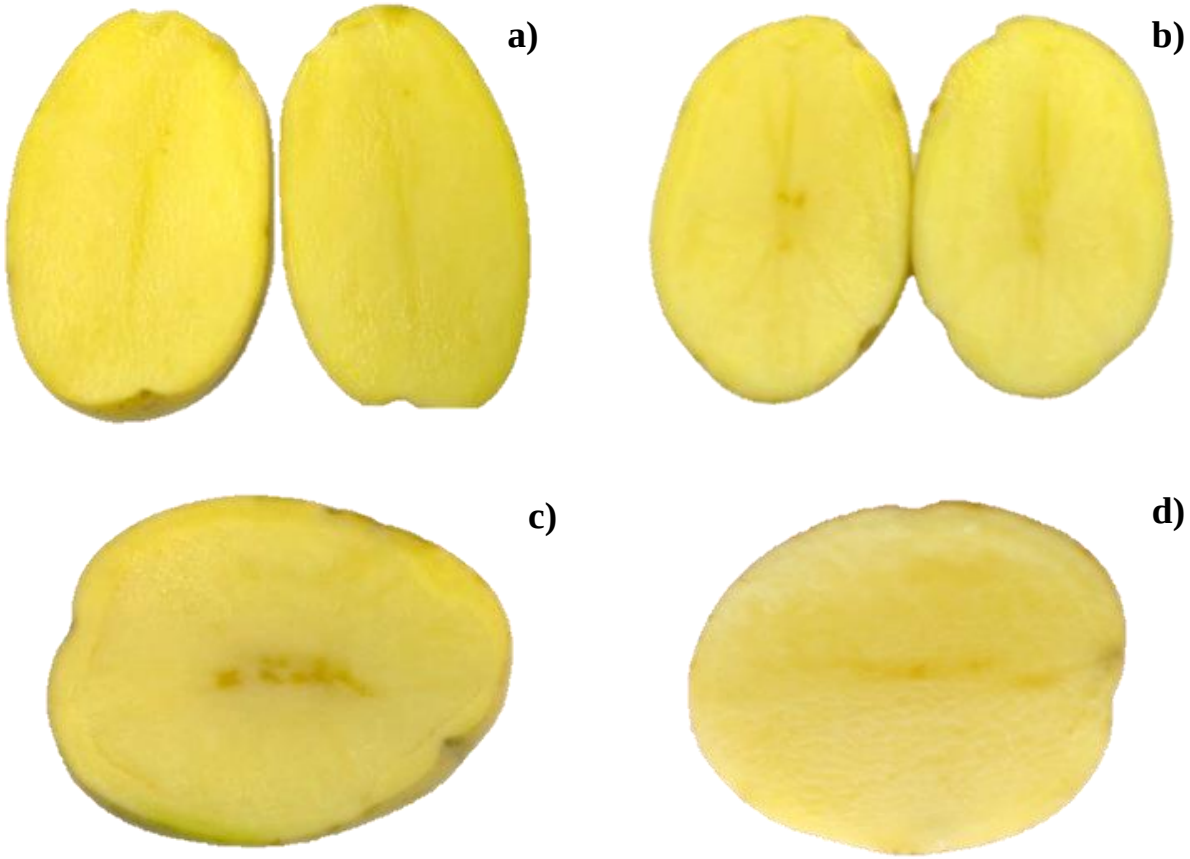

Figure 1.2 Example of BH incidence of potato tubers of stock 23 (susceptible to BH) after storage in air (a, b) or CA (c, d) at 15^{o}C. Tubers were initially stored at 1.5^{o}C (a, c) or 3^{o}C (b, d) for 8 weeks.

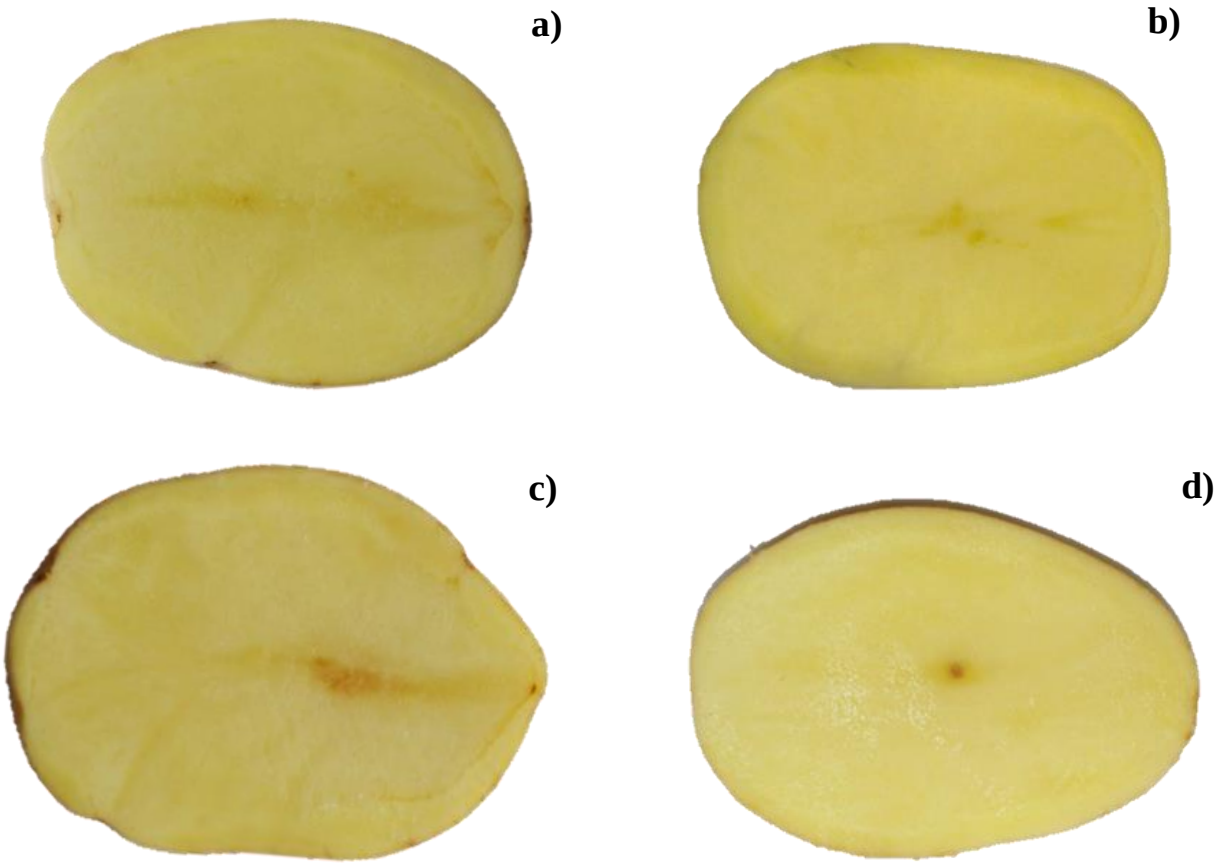

Figure 1.3 Example of BH incidence of potato tubers of stock 20 (susceptible to BH) after storage in air (a, b) or CA (c, d) at 15^{o}C. Tubers were initially stored at 1.5^{o}C (a, c) or 3^{o}C (b, d) for 12 weeks.

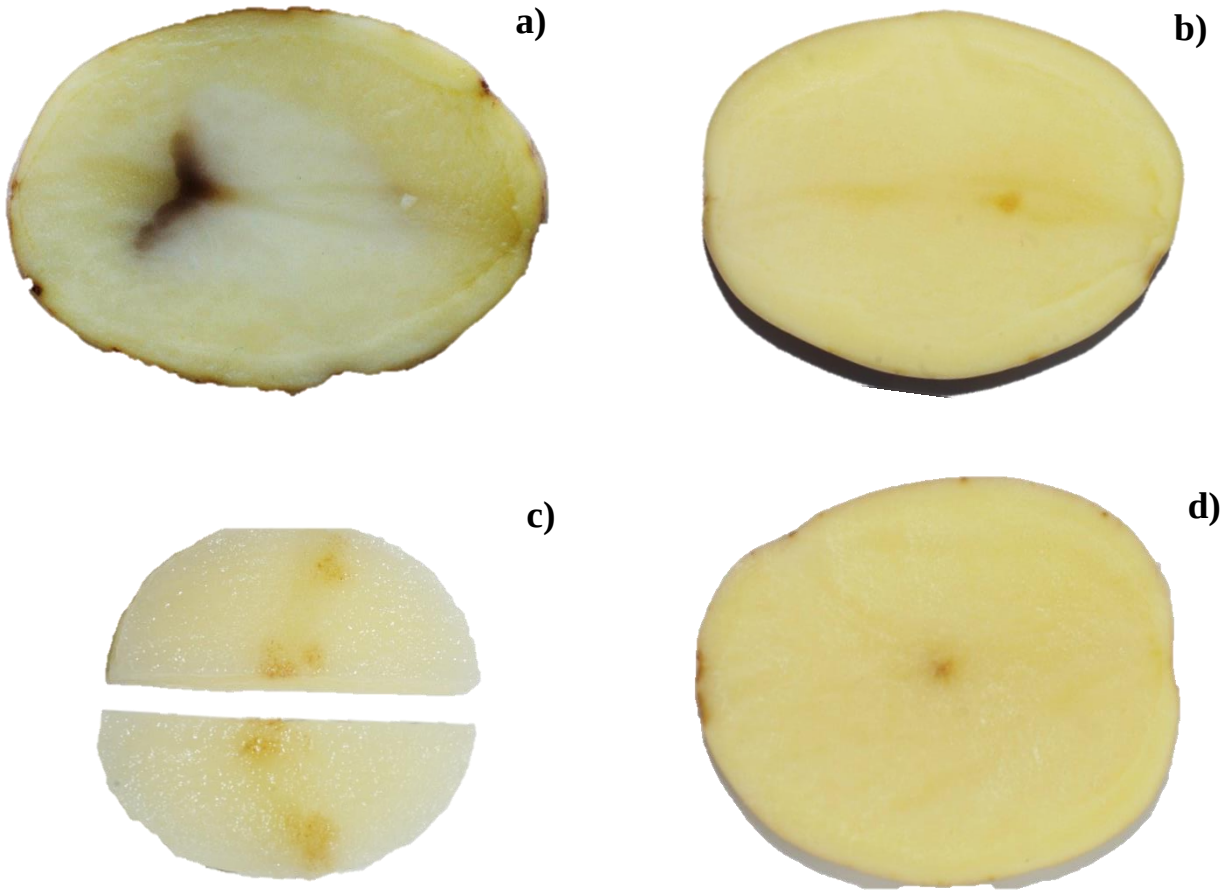

Figure 1.4 Example of BH incidence of potato tubers of stock 23 (susceptible to BH) after storage in air (a, b, c) or CA (d) at 15^{o}C. Tubers were initially stored at 1.5^{o}C (a, b, d) or 3^{o}C (c) for 12 weeks. Tuber heart part zoomed (c).

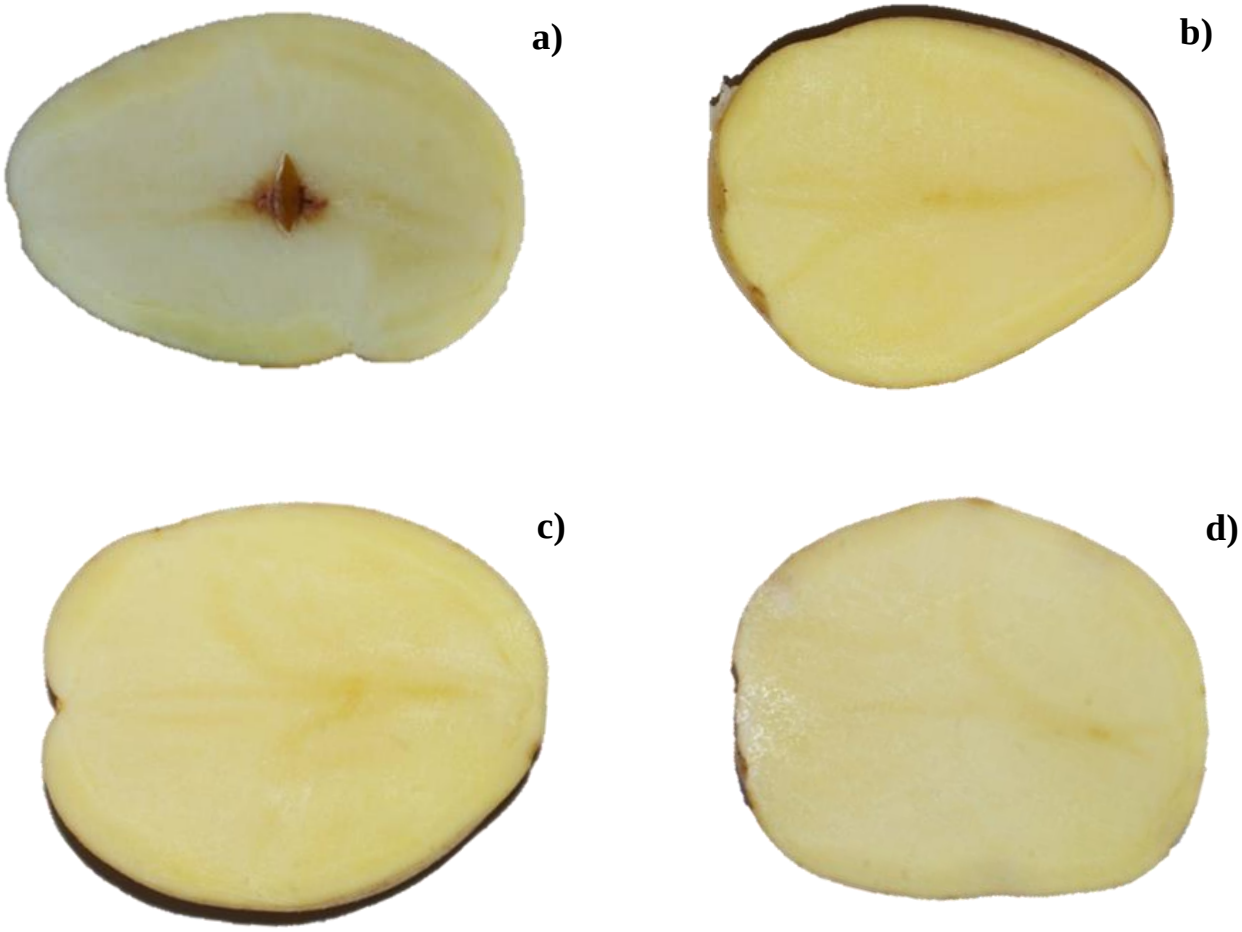

Figure 1.5 Example of BH incidence of potato tubers of stock 20 (susceptible to BH) after storage in air (a, b) or CA (c, d) at 15^{o}C. Tubers were initially stored at 1.5^{o}C (a, c, d) and 3^{o}C (b) for 16 weeks.

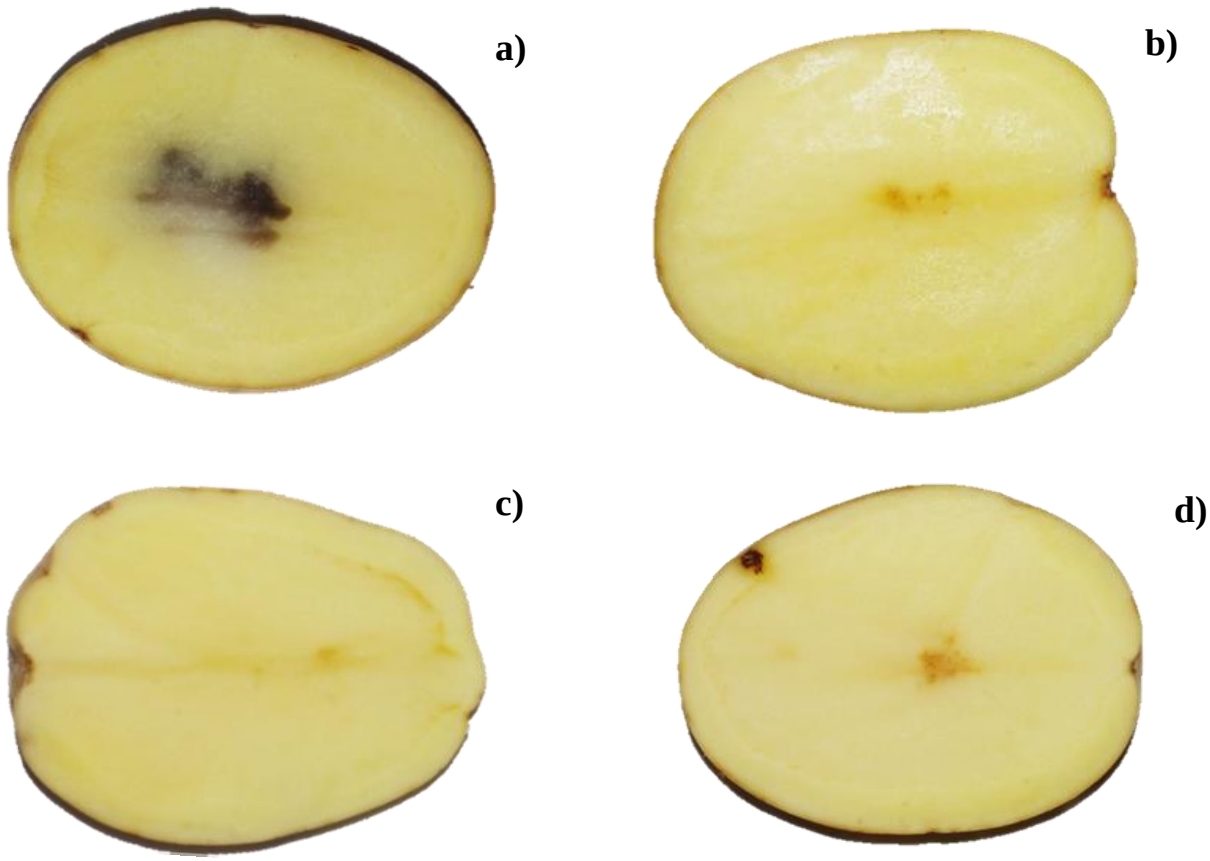

Figure 1.6 Example of BH incidence of potato tubers of stock 23 (susceptible to BH) after storage in air (a, b) or CA (c, d) at 15^{o}C. Tubers were initially stored at 1.5^{o}C (a, c) and 3^{o}C (b, d) for 16 weeks.

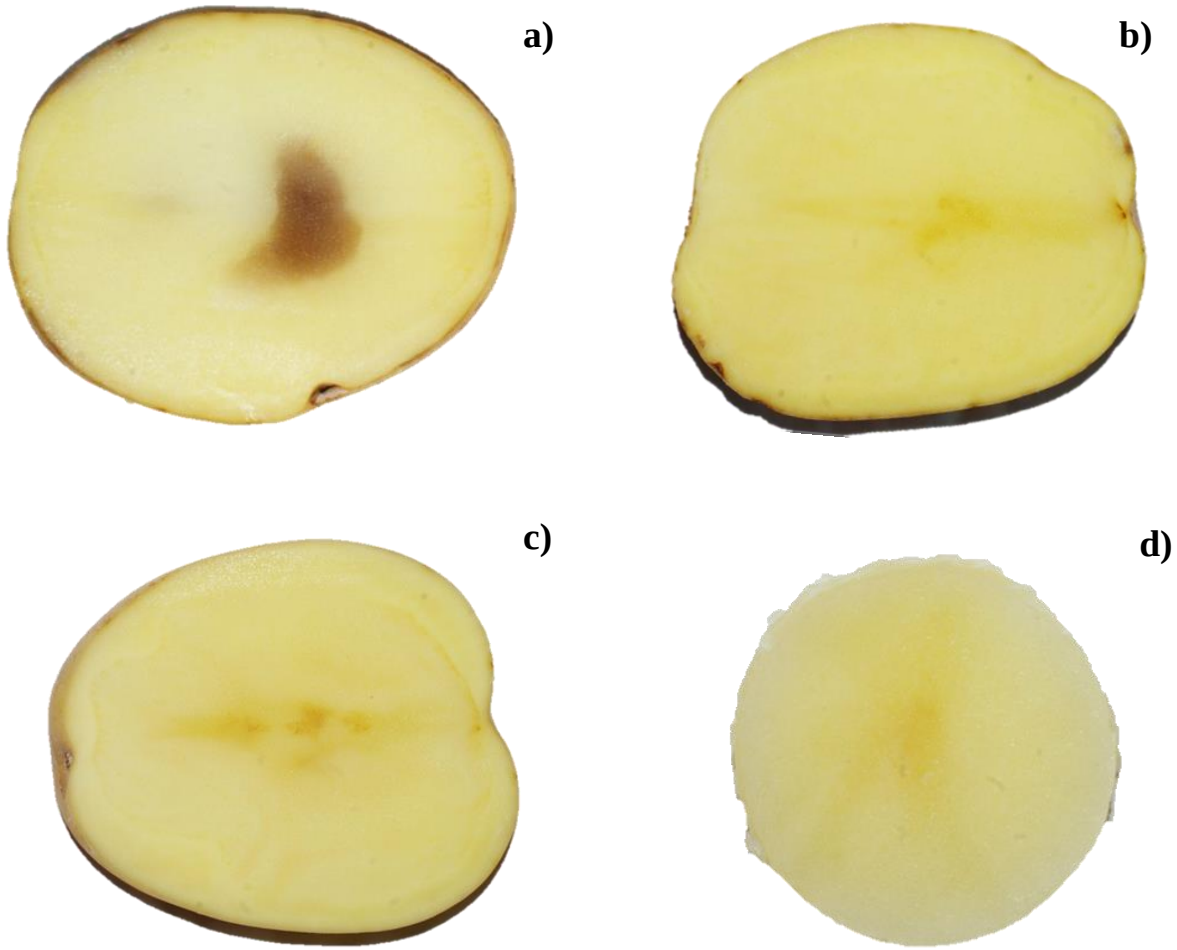

Figure 1.7 Example of BH incidence of potato tubers of stock 23 (susceptible to BH) after storage in air (a, b) or CA (c, d) at 15^{o}C. Tubers were initially stored at 1.5^{o}C (a, c) and 3^{o}C (b, d) for 24 weeks. Tube heart part zoomed (d).

2. Example of blackheart (BH) incidence of potato tubers in year 2 (2012-2013).

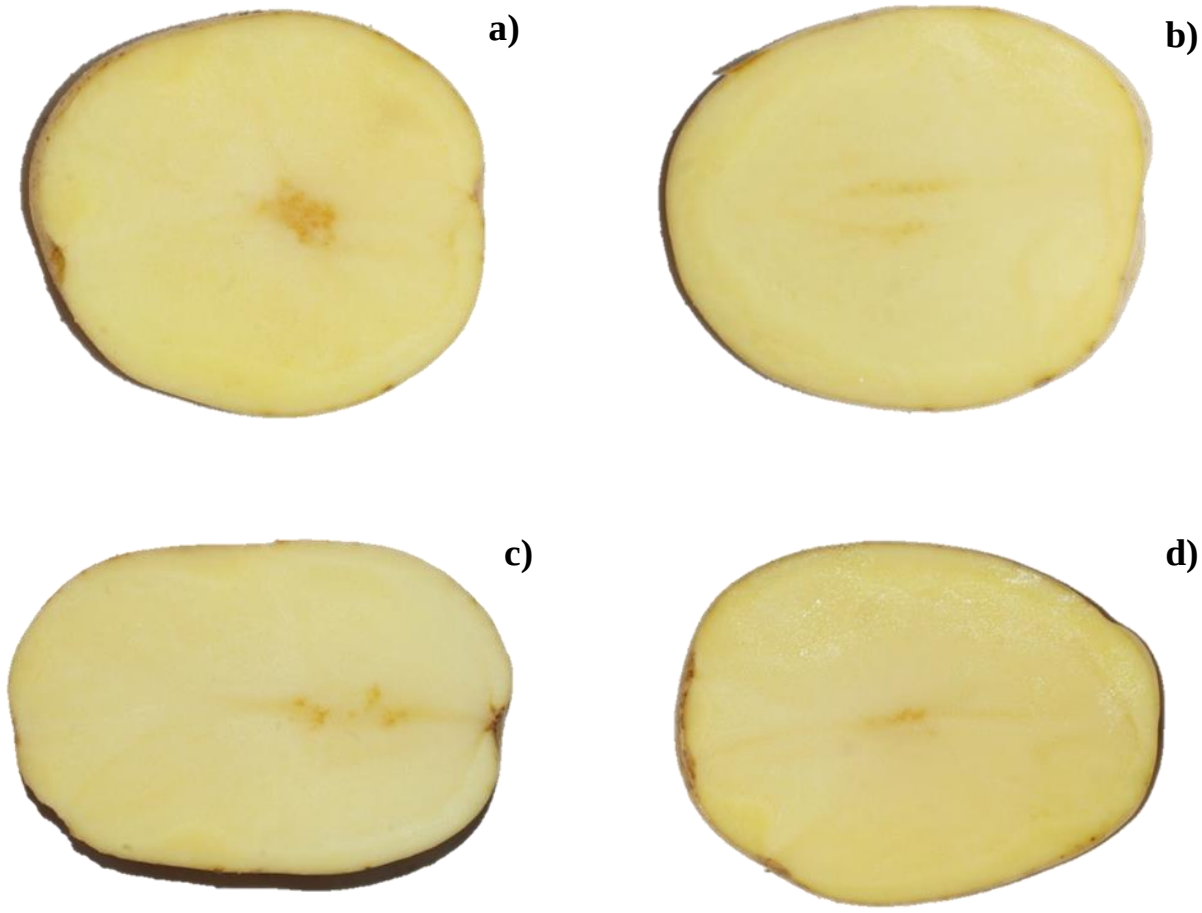

Figure 2.1 Example of BH incidence of potato tubers of stock 7 (susceptible to BH) (a) and stock 12 (susceptible to BH) (b) after baseline in air storage at 20^{o}C on sampling day 7.

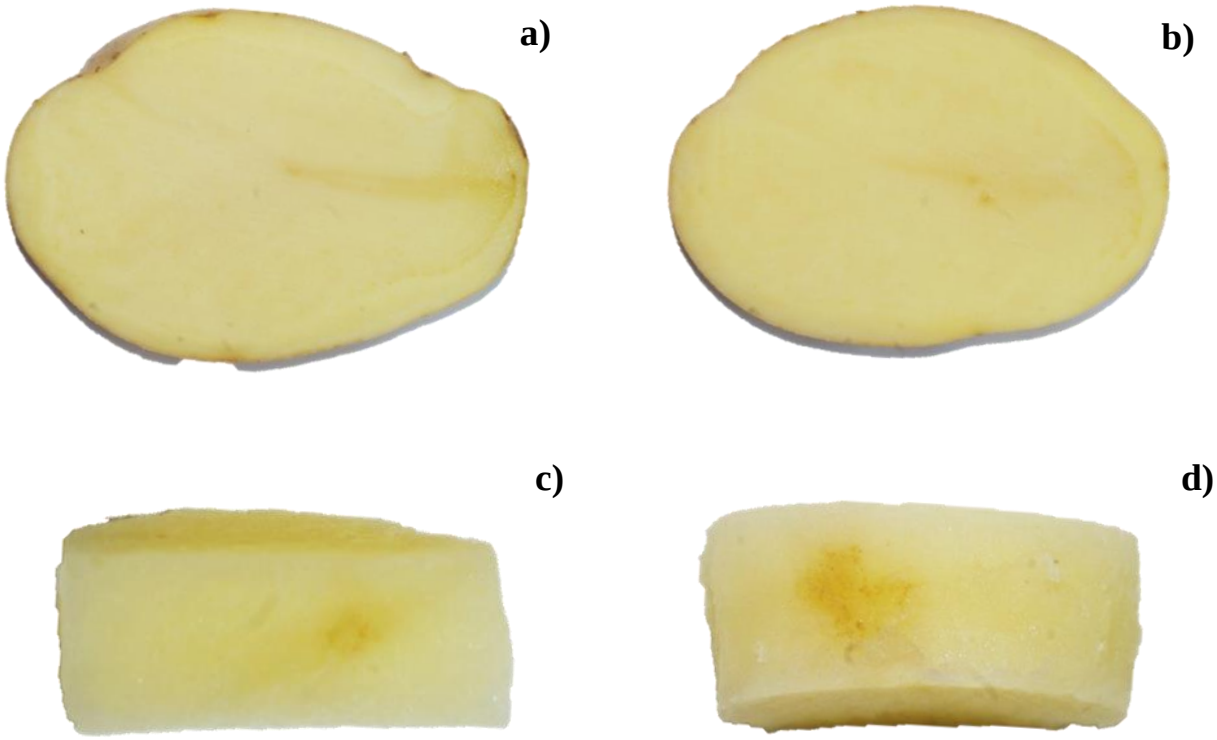

Figure 2.2 Example of BH incidence of potato tubers of stock 7 (susceptible to BH) (a, b) and stock 12 (susceptible to BH) (c, d) after storage in air at 20^{o}C on sampling day 0 (a, c) and day 7 (b, d). Tubers were initially stored at 1.5^{o}C for 4 weeks. Tuber heart part zoomed (c, d).

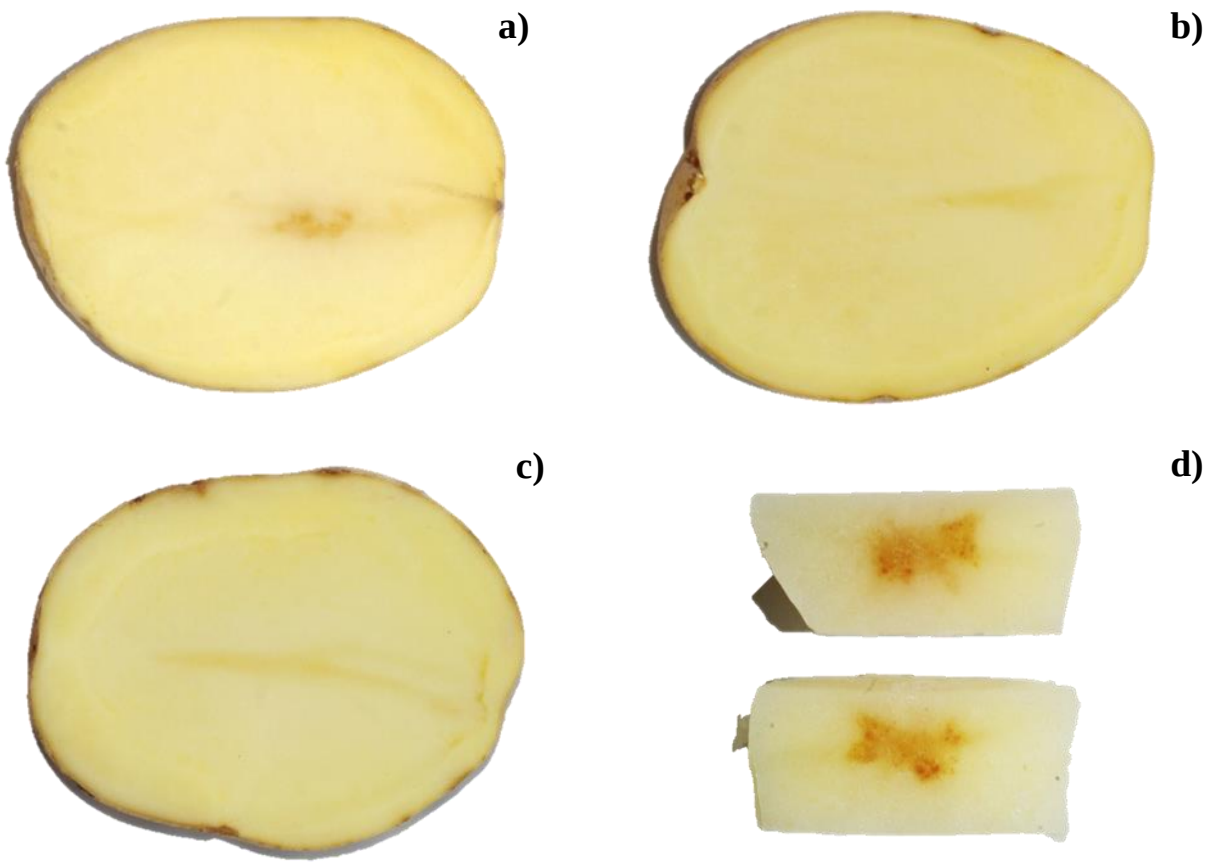

Figure 2.3 Example of BH incidence of potato tubers of stock 7 (susceptible to BH) (a, b) and stock 12 (susceptible to BH) (c, d) after storage in air at 20^{o}C on sampling day 0 (a, c, d) and day 7 (b). Tubers were initially stored at 1.5^{o}C for 12 weeks. Tuber heart part zoomed (d).

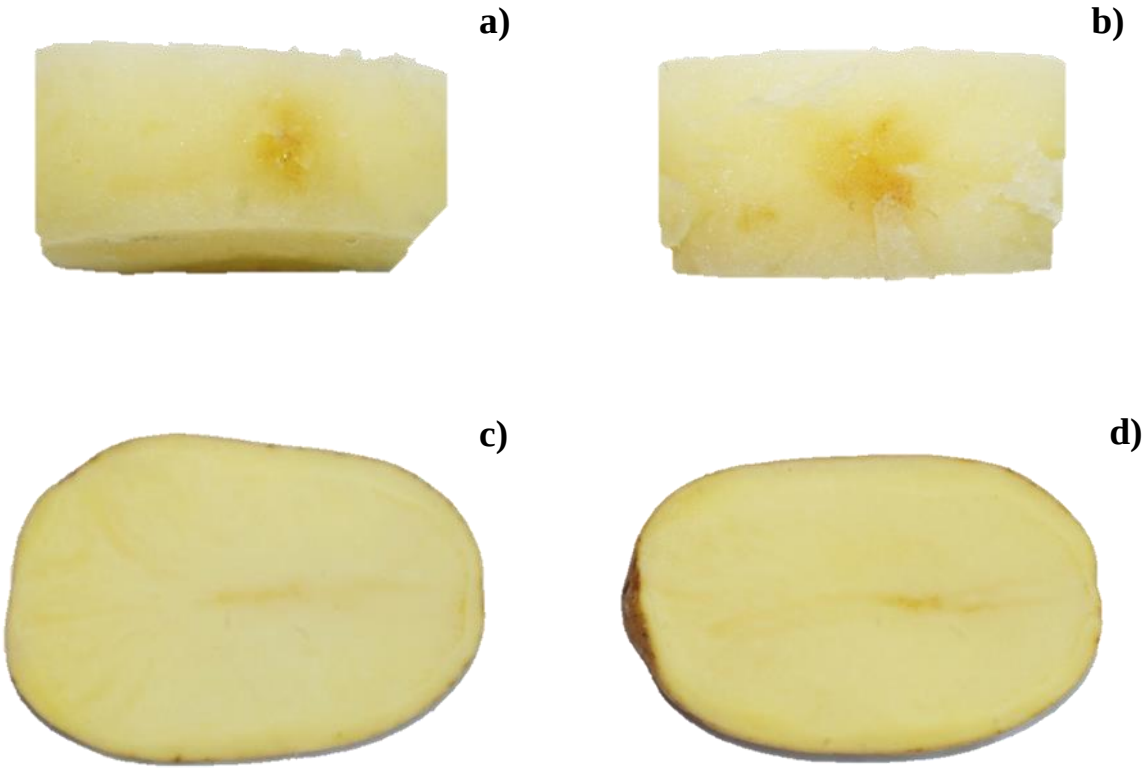

Figure 2.4 Example of BH incidence of potato tubers of stock 7 (susceptible to BH) (a, b) and stock 12 (susceptible to BH) (c, d) after storage in air at 20^{o}C on sampling day 0 (a, c) and day 7 (b, d). Tubers were initially stored at 1.5^{o}C for 16 weeks. Tuber heart part zoomed (a, b).

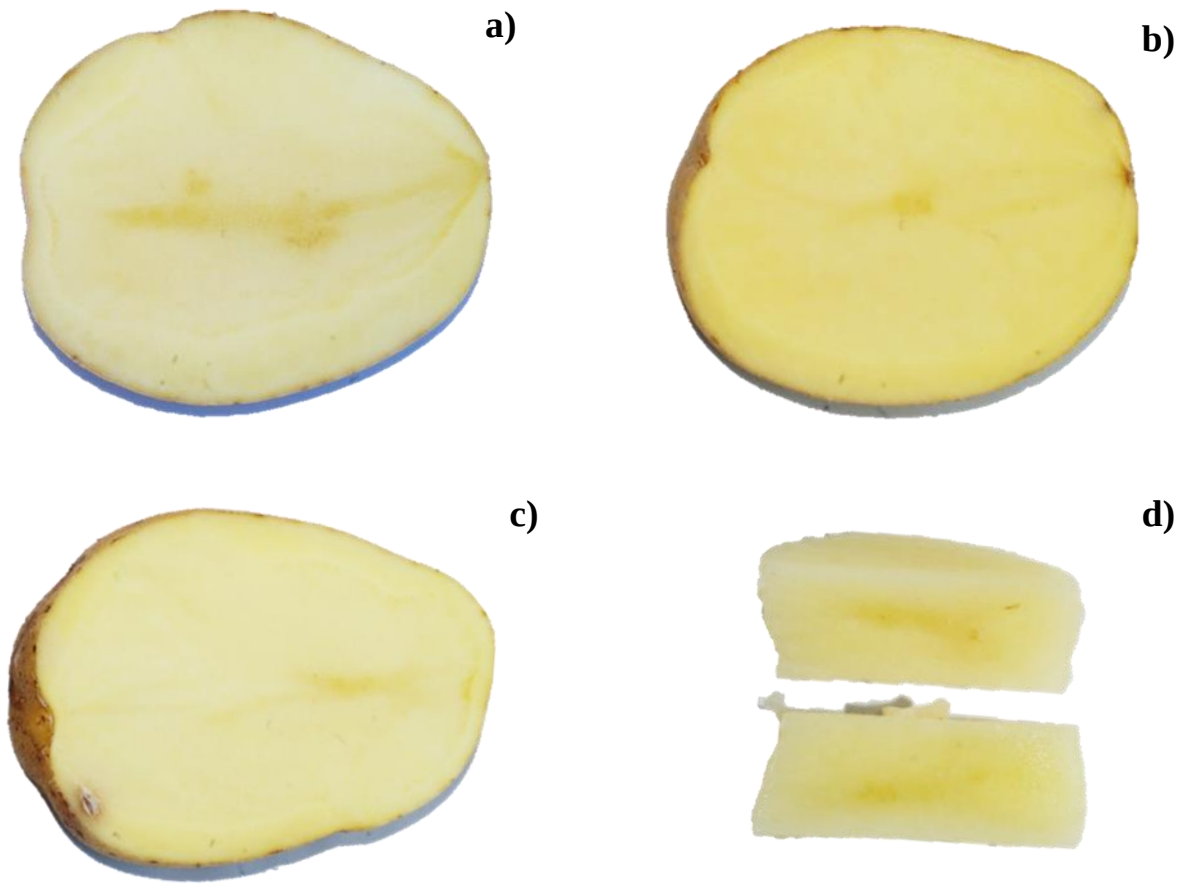

Figure 2.5 Example of BH incidence of potato tubers of stock 7 (susceptible to BH) (a, b) and stock 12 (susceptible to BH) (c, d) after storage in air at 20^{o}C on sampling day 0 (a, c) and day 7 (b, d). Tubers were initially stored at 1.5^{o}C for 20 weeks. Tuber heart part zoomed (d).

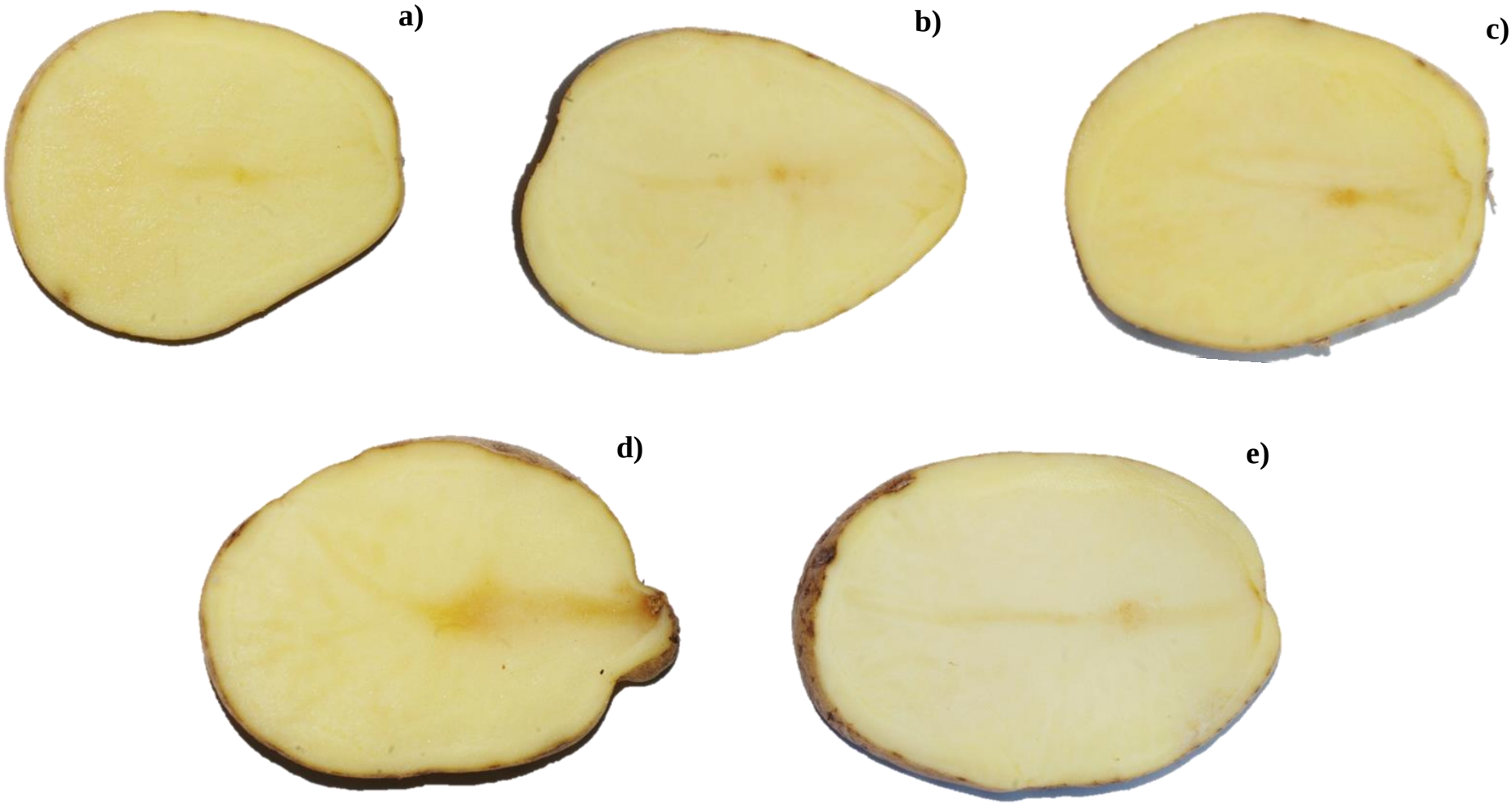

Figure 2.6 Example of BH incidence of potato tubers of stock 7 (susceptible to BH) after storage in 10% CO_2 (a), 10% O_2 (b) and 5% O_2 (c) and stock 12 (susceptible to BH) after storage in 10% CO_2 (d) and 5% O_2 (e) at 20^{o}C on sampling day 3. Tubers were initially stored at 1.5^{o}C for ca. 8 months.

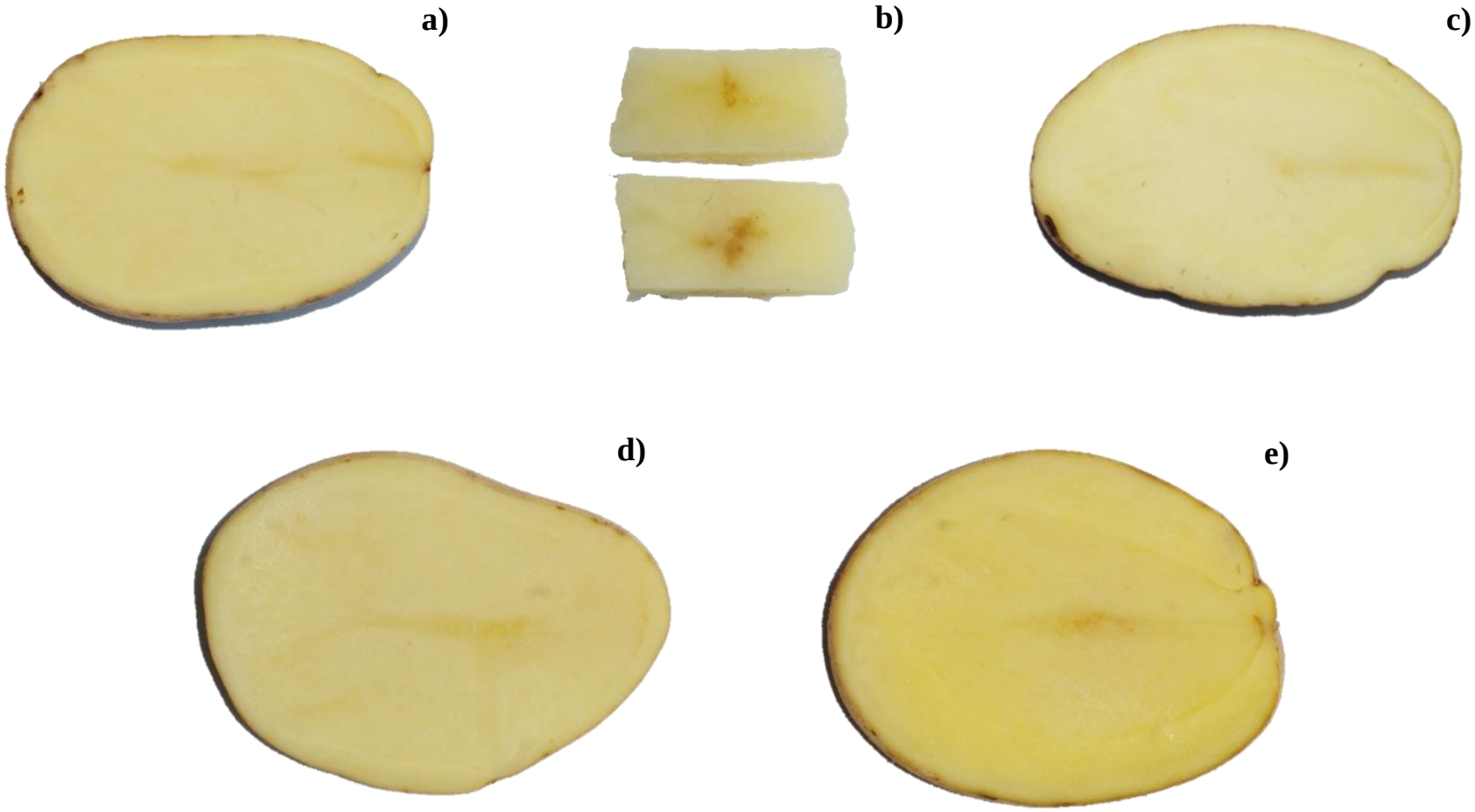

Figure 2.7 Example of BH incidence of potato tubers of stock 12 (susceptible to BH) after storage in 10% CO_2 (a), 10% O_2 (b) and 5% O_2 (c) and stock 12 (susceptible to BH) after storage in 10% CO_2 (d) and 5% O_2 (e) at 20^{o}C on sampling day 10. Tubers were initially stored at 1.5^{o}C for ca. 8 months. Tuber heart part zoomed (b).

Appendix B)

1. Analysis of Variance (ANOVA) for respiration rate of potato cv. Maris Piper stock 20 (susceptible to BH), stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) recorded after storage at 15^{o}C on sampling days 0 (air only), 3 (air or CA) and 7 (air or CA). Tubers were initially stored at 1.5 or 3^{o}C for 8, 12, 16 and 20 weeks. Structure used: Stock*CONDCODE/(Condition*TEMPCODE/(temp*OT*days))

Variate: Year 1 - CO_2

Source of variation	d.f.	(m.v.)	s.s	m.s.	v.r.	Fp.
Stock	2		236.439	118.219	52.35	< 0.001
CONDCODE	1		2118.139	2118.139	938.01	< 0.001
Stock.CONDCODE	2		61.098	30.549	13.53	< 0.001
CONDCODE.Condition	1		3601.92	3601.92	1595.12	< 0.001
CONDCODE.TEMPCODE	2		44.288	22.144	9.81	< 0.001
Stock.CONDCODE.Condition	2		94.712	47.536	20.97	< 0.001
Stock.CONDCODE.TEMPCODE	4		36.969	9.242	4.09	0.003
CONDCODE.Condition.TEMPCODE	1		0.496	0.496	0.22	0.640
CONDCODE.TEMPCODE.Temp	2		653.223	326.611	144.64	< 0.001
CONDCODE.TEMPCODE.OT	6		103.148	17.191	7.61	< 0.001
CONDCODE.TEMPCODE.Days	2		3.720	1.860	0.82	0.440

Stock.CONDCODE.Condition.TEMPCODE	2	0.444	0.222	0.10	0.906
Stock.CONDCODE.TEMPCODE.Temp	4	5.009	1.252	0.55	0.609
CONDCODE.Condition.TEMPCODE.Temp	1	0.078	0.078	0.03	0.852
Stock.CONDCODE.TEMPCODE.OT	12	34.772	2.898	1.28	0.228
CONDCODE.Condition.TEMPCODE.OT	3	129.649	43.216	19.14	< 0.001
CONDCODE.TEMPCODE.Temp.OT	6	14.062	2.344	1.04	0.401
Stock.CONDCODE.TEMPCODE.Days	4	12.755	3.189	1.41	0.230
CONDCODE.Condition.TEMPCODE.Days	2	6.133	8.067	3.57	0.020
CONDCODE.TEMPCODE.Temp.Days	1	24.214	24.214	10.72	0.001
CONDCODE.TEMPCODE.OT.Days	3	100.161	33.397	14.79	< 0.001
Stock.CONDCODE.Condition.TEMPCODE.Temp	2	4.342	2.171	0.96	0.384
Stock.CONDCODE.Condition.TEMPCODE.OT	6	29.760	4.960	2.20	0.044
Stock.CONDCODE.TEMPCODE.Temp.OT	12	9.966	0.831	0.37	0.974
CONDCODE.Condition.TEMPCODE.Temp.OT	3	3.177	1.059	0.47	0.704
Stock.CONDCODE.Condition.TEMPCODE.Days	4	21.694	5.424	2.40	0.050
Stock.CONDCODE.TEMPCODE.Temp.Days	2	4.860	2.430	1.08	0.342
CONDCODE.Condition.TEMPCODE.Temp.Days	1	0.128	0.128	0.06	0.812
Stock.CONDCODE.TEMPCODE.OT.Days	6	18.304	3.051	1.35	0.235
CONDCODE.Condition.TEMPCODE.OT.Days	3	47.440	15.813	7.00	< 0.001
CONDCODE.TEMPCODE.Temp.OT.Days	3	45.952	15.317	6.78	< 0.001
Stock.CONDCODE.Condition.TEMPCODE.Temp.OT	6	2.219	0.370	0.16	0.986

Stock.CONDCODE.Condition.TEMPCODE.Temp.Days	2		14.617	7.339	3.25	0.040
Stock.CONDCODE.Condition.TEMPCODE.OT.Days	6		32.715	5.452	2.41	0.027
Stock.CONDCODE.TEMPCODE.Temp.OT.Days	6		16.833	2.805	1.24	0.285
CONDCODE.Condition.TEMPCODE.Temp.OT.Days	3		12.457	4.152	1.84	0.140
Stock.CONDCODE.Condition.TEMPCODE.Temp.OT.Days	6		7.532	1.255	0.56	0.765
Residual	326	(8)	591.625			
Total	396	(8)	7650.236			

2. Chi-square test results of BH incidence for potato tubers cv. Maris Piper stock 20 and stock 23 after storage in air or CA at 15°C. Tubers were initially stored at 1.5 or 3°C. (*P* < 0.05)

Table 2.1 Chi-square test results of BH susceptibility between stock 20 and stock 23 (both susceptible to BH) after storage in air at 15°C. Tubers were initially stored at 1.5°C.

	OBSERVED	BH	NO BH	TOTAL
	Stock 20	6	102	108
	Stock 23	22	86	108
		28	188	216
	EXPECTED	BH	NO BH	TOTAL
	Stock 20	14	94	108
	Stock 23	14	94	108
		28	188	216
p=	0.001190804			

Table 2.2 Chi-square test results of BH susceptibility between stock 20 and stock 23 (both susceptible to BH) after storage in air at 15°C. Tubers were initially stored at 3°C.

	OBSERVED	BH	NO BH	TOTAL
	Stock 20	8	100	108
	Stock 23	22	86	108
		30	188	216
	EXPECTED	BH	NO BH	TOTAL
	Stock 20	15	93	108
	Stock 23	15	93	108
		30	186	216
p=	0.005878755			

Table 2.3 Chi-square test results of BH susceptibility between stock 20 and stock 23 (both susceptible to BH) after storage in CA at 15°C. Tubers were initially stored at 1.5°C.

	OBSERVED	BH	NO BH	TOTAL
	Stock 20	4	68	72
	Stock 23	7	65	72
		11	133	144
	EXPECTED	BH	NO BH	TOTAL
	Stock 20	5.5	66.5	72
	Stock 23	5.5	66.5	72

		11	133	144
p=	0.013603695			

Table 2.4 Chi-square test results of BH susceptibility between stock 20 and stock 23 (both susceptible to BH) after storage in CA at 15°C. Tubers were initially stored at 3°C.

OBSERVED	BH	NO BH	TOTAL
Stock 20	3	69	72
Stock 23	11	61	72
	14	130	144
EXPECTED	BH	NO BH	TOTAL
Stock 20	7	65	72
Stock 23	7	65	72
	14	130	144
p=	0.024431512		

3. Two-sample t-test results of tuber length, diameter and weight for potato cv. Maris Piper stock 20 (susceptible to BH) after storage in air at 15°C. Tubers were initially stored at 1.5 or 3°C for 8, 12, 16 and 20 weeks.

Table 3.1 Two-sample t-test results of tuber length (mm) for potato cv. Maris Piper stock 20 (susceptible to BH) in air at 15°C. Tubers were initially stored at 3°C for 8 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	23	98.83	106.34	10.312	2.150
Affected	4	93.99	85.67	9.256	4.628
Probability = 0.805					

Table 3.2 Two-sample t-test results of tuber diameter (mm) for potato cv. Maris Piper stock 20 (susceptible to BH) in air at 15°C. Tubers were initially stored at 3°C for 8 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	23	72.96	27.57	5.251	1.095
Affected	4	72.59	16.07	4.009	2.004
Probability = 0.552					

Table 3.3 Two-sample t-test results of tuber weight (g) for potato cv. Maris Piper stock 20 (susceptible to BH) in air at 15°C. Tubers were initially stored at 3°C for 8 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	23	248.6	2429	49.29	10.277
Affected	4	231.9	213	14.59	7.294

Probability = 0.743

Table 3.4 Two-sample t-test results of tuber length (mm) for potato cv. Maris Piper stock 20 (susceptible to BH) in air at 15°C. Tubers were initially stored at 1.5°C for 12 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	25	92.74	133.06	11.535	2.307
Affected	2	96.36	1.36	1.167	0.825

Probability = 0.334

Table 3.5 Two-sample t-test results of tuber diameter (mm) for potato cv. Maris Piper stock 20 (susceptible to BH) in air at 15°C. Tubers were initially stored at 1.5°C for 12 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	25	70.44	36.89	6.073	1.215
Affected	2	70.39	54.71	7.396	5.230

Probability = 0.505

Table 3.6 Two-sample t-test results of tuber weight (g) for potato cv. Maris Piper stock 20 (susceptible to BH) in air at 15°C. Tubers were initially stored at 1.5°C for 12 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	25	218.5	3859	62.12	12.42
Affected	2	234.2	384	19.59	13.85

Probability = 0.365

Table 3.7 Two-sample t-test results of tuber length (mm) for potato cv. Maris Piper stock 20 (susceptible to BH) in air at 15°C. Tubers were initially stored at 1.5°C for 16 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	25	87.06	102.76	10.137	2.027
Affected	2	104.90	17.88	4.228	2.990

Probability = 0.011

Table 3.8 Two-sample t-test results of tuber diameter (mm) for potato cv. Maris Piper stock 20 (susceptible to BH) in air at 15°C. Tubers were initially stored at 1.5°C for 16 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	25	69.60	30.31	5.506	1.101
Affected	2	78.78	19.41	4.405	3.115

Probability = 0.016

Table 3.9 Two-sample t-test results of tuber weight (g) for potato cv. Maris Piper stock 20 (susceptible to BH) in air at 15°C. Tubers were initially stored at 1.5°C for 16 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	25	198.7	2020	44.94	8.99
Affected	2	313.2	272	16.50	11.66

Probability < 0.001

Table 3.10 Two-sample t-test results of tuber length (mm) for potato cv. Maris Piper stock 20 (susceptible to BH) in air at 15°C. Tubers were initially stored at 3 °C for 16 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	25	96.49	111.8	10.58	2.115
Affected	2	100.08	190.7	13.81	9.765

Probability = 0.326

Table 3.11 Two-sample t-test results of tuber diameter (mm) for potato cv. Maris Piper stock 20 (susceptible to BH) in air at 15°C. Tubers were initially stored at 3°C for 16 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	25	72.57	31.24	5.590	1.118
Affected	2	75.32	34.61	5.883	4.160

Probability = 0.255

Table 3.12 Two-sample t-test results of tuber weight (g) for potato cv. Maris Piper stock 20 (susceptible to BH) in air at 15°C. Tubers were initially stored at 3°C for 16 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	25	231.5	2570	50.70	10.14
Affected	2	253.2	3515	59.28	41.92

Probability = 0.284

Table 3.13 Correlation comparing the tuber length, diameter and weight with the BH incidence for potato cv. Maris Piper stock 20 after storage in air at 15°C. Tubers were initially stored at 1.5°C for 16 weeks.

BH	1	-			
Diameter	2	0.4155	-		
Length	3	0.4378	0.4716	-	
Weight	4	0.5765	0.7298	0.8671	-
		1	2	3	4

Two-sided test of correlations different from zero

BH	1	-			
Diameter	2	0.0312	-		
Length	3	0.0224	0.0130	-	
Weight	4	0.0016	<0.001	<0.001	-
		1	2	3	4

4. Two-sample t-test results of tuber length, diameter and weight with BH incidence for potato cv. Maris Piper stock 20 (susceptible to BH) after storage in CA at 15°C. Tubers were initially stored at 1.5 or 3°C for 8, 12, 16 and 20 weeks.

Table 4.1 Two-sample t-test results of tuber length (mm) with BH incidence for potato cv. Maris Piper stock 20 (susceptible to BH) in CA at 15°C. Tubers were initially stored at 1.5°C for 8 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	87.82	103.5	10.17	2.544
Affected	2	103.30	219.0	14.80	10.465

Probability = 0.034

Table 4.2 Two-sample t-test results of tuber diameter (mm) with BH incidence for potato cv. Maris Piper stock 20 (susceptible to BH) in CA at 15°C. Tubers were initially stored at 1.5°C for 8 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	68.90	79.4	8.91	2.227
Affected	2	73.91	156.5	12.51	8.845

Probability = 0.238

Table 4.3 Two-sample t-test results of tuber weight (g) with BH incidence for potato cv. Maris Piper stock 20 (susceptible to BH) in CA at 15°C. Tubers were initially stored at 1.5°C for 8 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	214.5	2277	47.72	11.93
Affected	2	262.4	21210	145.64	102.98

Probability = 0.361

Table 4.4 Two-sample t-test results of tuber length (mm) with BH incidence for potato cv. Maris Piper stock 20 (susceptible to BH) in CA at 15°C. Tubers were initially stored at 3°C for 8 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	92.18	72.92	8.539	2.135
Affected	2	96.95	102.24	10.112	7.150

Probability = 0.236

Table 4.5 Two-sample t-test results of tuber diameter (mm) with BH incidence for potato cv. Maris Piper stock 20 (susceptible to BH) in CA at 15°C. Tubers were initially stored at 3°C for 8 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	68.88	26.45	5.143	1.286
Affected	2	70.75	78.75	8.874	6.275

Probability = 0.327

Table 4.6 Two-sample t-test results of tuber weight (g) with BH incidence for potato cv. Maris Piper stock 20 (susceptible to BH) in CA at 15°C. Tubers were initially stored at 3°C for 8 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	225.8	1358.6	36.86	9.21
Affected	2	236.8	617.1	24.84	17.56

Probability = 0.346

Table 4.7 Correlation matrix comparing the tuber length, diameter and weight with the BH incidence for potato cv. Maris Piper stock 20 (susceptible to BH) after storage in CA at 15°C. Tubers were initially stored at 1.5°C for 8 weeks.

BH	1	-			
Diameter	2	0.1792	-		
Length	3	0.4402	0.7400	-	
Weight	4	0.2620	0.8578	0.8173	-
		1	2	3	4

Two-sided test of correlations different from zero					
BH	1	-			
Diameter	2	0.4769	-		
Length	3	0.0675	<0.001	-	
Weight	4	0.2935	<0.001	<0.001	-
		1	2	3	4

5. Two-sample t-test results of tuber length, diameter and weight for potato cv. Maris Piper stock 23 after storage in air at 15^{o}C.Tubers were initially stored at 1.5 or 3^{o}C for 8, 12, 16 and 20 weeks.

Table 5.1 Two-sample t-test of tuber length (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15^{o}C. Tubers were initially stored at 1.5^{o}C for 8 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	25	101.9	162.3	12.74	2.548
Affected	2	109.1	234.1	15.30	10.820

Probability = 0.227

Table 5.2 Two-sample t-test of tuber diameter (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15^{o}C. Tubers were initially stored at 1.5^{o}C for 8 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	25	75.62	30.1	5.488	1.098
Affected	2	75.68	189.7	13.774	9.740

Probability = 0.498

Table 5.3 Two-sample t-test results of tuber weight (g) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15°C. Tubers were initially stored at 1.5°C for 8 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	25	281.6	4441	66.64	13.33
Affected	2	284.3	17421	131.99	93.33

Probability = 0.480

Table 5.4 Two-sample t-test results of tuber length (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15°C. Tubers were initially stored at 3°C for 8 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	23	99.82	56.12	7.492	1.562
Affected	4	98.59	85.38	9.240	4.620

Probability = 0.614

Table 5.5 Two-sample t-test results of tuber diameter (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15°C. Tubers were initially stored at 3°C for 8 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	23	72.95	34.51	5.874	1.225
Affected	4	74.29	10.40	3.226	1.613

Probability = 0.331

Table 5.6 Two-sample t-test results of tuber weight (g) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15°C. Tubers were initially stored at 3°C for 8 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	23	254.9	2713	52.09	10.86
Affected	4	274.1	1658	40.71	20.36

Probability = 0.247

Table 5.7 Two-sample t-test results of tuber length (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15°C. Tubers were initially stored at 1.5°C for 12 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	24	96.25	101.5	10.07	2.056
Affected	3	97.08	277.6	16.66	9.620

Probability = 0.450

Table 5.8 Two-sample t-test results of tuber diameter (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15°C. Tubers were initially stored at 1.5°C for 12 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	24	73.22	19.35	4.399	0.898
Affected	3	69.19	46.86	6.845	3.952

Probability = 0.916

Table 5.9 Two-sample t-test results of tuber weight (g) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15°C. Tubers were initially stored at 1.5°C for 12 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	24	245.2	2751	52.45	10.71
Affected	3	221.2	7958	89.21	51.50

Probability = 0.754

Table 5.10 Two-sample t-test results of tuber length (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15°C. Tubers were initially stored at 3°C for 12 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	23	91.31	121.05	11.002	2.294
Affected	4	95.55	16.72	4.089	2.045

Probability = 0.230

Table 5.11 Two-sample t-test results of tuber diameter (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15°C. Tubers were initially stored at 3°C for 12 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	23	73.13	28.17	5.307	1.107
Affected	4	70.17	42.79	6.542	3.271

Probability = 0.836

Table 5.12 Two-sample t-test results of tuber weight (g) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15°C. Tubers were initially stored at 3°C for 12 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	23	231.5	2952	54.34	11.33
Affected	4	235.5	3783	61.51	30.75

Probability = 0.447

Table 5.13 Two-sample t-test results of tuber length (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15°C. Tubers were initially stored at 1.5°C for 16 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	17	97.25	84.46	9.190	2.229
Affected	10	94.40	90.29	9.502	3.005

Probability = 0.775

Table 5.14 Two-sample t-test results of tuber diameter (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15^{o}C. Tubers were initially stored at 1.5^{o}C for 16 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	17	75.04	24.13	4.913	1.191
Affected	10	74.15	25.12	5.012	1.585

Probability = 0.672

Table 5.15 Two-sample t-test results of tuber weight (g) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15^{o}C. Tubers were initially stored at 1.5^{o}C for 16 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	17	251.7	1455	38.14	9.25
Affected	10	244.2	3137	56.01	17.71

Probability = 0.659

Table 5.16 Two-sample t-test results of tuber length (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15^{o}C. Tubers were initially stored at 3^{o}C for 16 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	18	93.31	53.85	7.339	1.730
Affected	9	94.01	75.82	8.708	2.903

Probability = 0.414

Table 5.17 Two-sample t-test results of tuber diameter (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15°C. Tubers were initially stored at 3°C for 16 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	18	72.51	42.12	6.490	1.530
Affected	9	76.08	28.07	5.298	1.766

Probability = 0.083

Table 5.18 Two-sample t-test results of tuber weight (g) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15°C. Tubers were initially stored at 3°C for 16 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	18	238.8	2014	44.87	10.58
Affected	9	262.1	2625	51.23	17.08

Probability = 0.118

Table 5.19 Two-sample t-test results of tuber length (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15°C. Tubers were initially stored at 1.5°C for 20 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	20	96.58	181.5	13.47	3.012
Affected	7	92.43	76.4	8.74	3.304

Probability = 0.771

Table 5.20 Two-sample t-test results of tuber diameter (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15°C. Tubers were initially stored at 1.5°C for 20 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	20	70.16	41.79	6.465	1.446
Affected	7	73.49	29.46	5.428	2.052

Probability = 0.117

Table 5.21 Two-sample t-test results of tuber weight (g) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15^{o}C. Tubers were initially stored at 1.5^{o}C for 20 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	20	233.1	2978	54.57	12.20
Affected	7	235.3	2580	50.79	19.20

Probability = 0.463

Table 5.22 Two-sample t-test results of tuber length (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15^{o}C. Tubers were initially stored at 3^{o}C for 20 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	22	88.60	169.4	13.01	2.775
Affected	5	88.11	54.9	7.41	3.314

Probability = 0.531

Table 5.23 Two-sample t-test results of tuber diameter (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15^{o}C. Tubers were initially stored at 3^{o}C for 20 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	22	70.62	23.52	4.850	1.034
Affected	5	72.21	6.74	2.596	1.161

Probability = 0.244

Table 5.24 Two-sample t-test results of tuber weight (g) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in air at 15°C. Tubers were initially stored at 3°C for 20 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	22	211.8	3676	60.63	12.93
Affected	5	224.5	1030	32.10	14.35

Probability = 0.328

6. Two-sample t-test results of tuber length, diameter and weight for potato cv. Maris Piper stock 23 after storage in CA at 15°C. Tubers were initially stored at 1.5 or 3°C for 8, 12, 16 and 20 weeks.

Table 6.1 Two-sample t-test results of tuber length (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in CA at 15°C. Tubers were initially stored at 1.5°C for 8 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	13	98.31	148.0	12.16	3.374
Affected	5	98.65	138.5	11.77	5.263

Probability = 0.479

Table 6.2 Two-sample t-test results of tuber diameter (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in CA at 15°C. Tubers were initially stored at 1.5°C for 8 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	13	74.00	57.32	7.571	2.100
Affected	5	77.92	14.74	3.840	1.717

Probability = 0.146

Table 6.3 Two-sample t-test results of tuber weight (g) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in CA at 15^{o}C. Tubers were initially stored at 1.5^{o}C for 8 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	13	265.4	4416	66.45	18.43
Affected	5	272.7	1576	39.69	17.75

Probability = 0.411

Table 6.4 Two-sample t-test results of tuber length (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in CA at 15^{o}C. Tubers were initially stored at 3^{o}C for 8 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	98.7	164.40	12.822	3.205
Affected	2	104.0	13.16	3.627	2.565

Probability = 0.288

Table 6.5 Two-sample t-test results of tuber diameter (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in CA at 15^{o}C. Tubers were initially stored at 3^{o}C for 8 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	73.98	26.27	5.125	1.281
Affected	2	78.60	2.35	1.534	1.085

Probability = 0.117

Table 6.6 Two-sample t-test results of tuber weight (g) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in CA at 15^{o}C. Tubers were initially stored at 3^{o}C for 8 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	259.7	3869	62.21	15.55
Affected	2	286.9	703	26.52	18.75

Probability = 0.279

Table 6.7 Two-sample t-test results of tuber length (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in CA at 15^{o}C. Tubers were initially stored at 3^{o}C for 16 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	11	96.88	63.22	7.951	2.397
Affected	7	92.97	73.19	8.555	3.233

Probability = 0.831

Table 6.8 Two-sample t-test results of tuber diameter (mm) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in CA at 15^{o}C. Tubers were initially stored at 3^{o}C for 16 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	11	72.28	29.50	5.431	1.638
Affected	7	72.53	25.68	5.067	1.915

Probability = 0.463

Table 6.9 Two-sample t-test results of tuber weight (g) with BH incidence for potato cv. Maris Piper stock 23 (susceptible to BH) in CA at 15^{o}C. Tubers were initially stored at 3^{o}C for 16 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	11	242.4	1978	44.48	13.41
Affected	7	245.9	3171	56.31	21.28

Probability = 0.443

7. Analysis of variance (ANOVA) results of sugar content in potato tubers of year 1 (2011-2012). Structure used: STOCK*TISSUE*OT*DAY

Table 7.1 Variate: Year 1 - Fructose

Source of variation	d.f	s.s.	m.s.	v.r.	F pr.
STOCK	2	22117.95	11058.97	150.94	<.001
TISSUE	1	14695.45	14695.45	200.58	<.001
OT	3	54189.96	18063.32	246.54	<.001
DAY	2	2981.82	1490.91	20.35	<.001
STOCK.TISSUE	2	230.76	115.38	1.57	0.211
STOCK.OT	6	12649.41	2108.23	28.77	<.001
TISSUE.OT	3	2340.38	780.13	10.65	<.001
STOCK.DAY	4	144.63	36.16	0.49	0.741
TISSUE.DAY	2	194.45	97.23	1.33	0.268
OT.DAY	6	4634.17	772.36	10.54	<.001
STOCK.TISSUE.OT	6	590.42	98.40	1.34	0.242
STOCK.TISSUE.DAY	4	214.47	53.62	0.73	0.572
STOCK.OT.DAY	12	2553.09	212.76	2.90	0.001
TISSUE.OT.DAY	6	1004.86	167.48	2.29	0.039
STOCK.TISSUE.OT.DAY	12	688.26	57.36	0.78	0.667
Residual	144	10550.38	73.27		
Total	215	129780.45			

Table 7.2 Variate: Year 1 - Glucose

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
STOCK	2	30276.1	15138.0	142.85	<.001
TISSUE	1	13756.8	13756.8	129.82	<.001
OT	3	49989.8	16663.3	157.24	<.001
DAY	2	7946.7	3973.4	37.49	<.001
STOCK.TISSUE	2	365.7	182.9	1.73	0.182
STOCK.OT	6	26667.3	4444.5	41.94	<.001
TISSUE.OT	3	1390.6	463.5	4.37	0.006
STOCK.DAY	4	931.7	232.9	2.20	0.072
TISSUE.DAY	2	939.2	469.6	4.43	0.014
OT.DAY	6	6734.2	1122.4	10.59	<.001
STOCK.TISSUE.OT	6	775.6	129.3	1.22	0.299
STOCK.TISSUE.DAY	4	509.8	127.5	1.20	0.312
STOCK.OT.DAY	12	2957.6	246.5	2.33	0.009

TISSUE.OT.DAY	6	2473.3	412.2	3.89	0.001
STOCK.TISSUE.OT.DAY	12	1520.8	126.7	1.20	0.291
Residual	144	15259.8	106.0		
Total	215	162495.2			

Table 7.3 Variate: Year 1 – Sucrose

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
STOCK	2		1090.607	545.303	61.75	<.001
TISSUE	1		582.034	582.034	65.91	<.001
OT	3		364.974	121.658	13.78	<.001
DAY	2		919.555	459.778	52.06	<.001
STOCK.TISSUE	2		87.697	43.849	4.97	0.008
STOCK.OT	6		761.463	126.911	14.37	<.001
TISSUE.OT	3		70.280	23.427	2.65	0.051
STOCK.DAY	4		26.177	6.544	0.74	0.566
TISSUE.DAY	2		27.080	13.540	1.53	0.219
OT.DAY	6		342.644	57.107	6.47	<.001
STOCK.TISSUE.OT	6		351.302	58.550	6.63	<.001
STOCK.TISSUE.DAY	4		37.043	9.261	1.05	0.384
STOCK.OT.DAY	12		101.908	8.492	0.96	0.488
TISSUE.OT.DAY	6		54.729	9.122	1.03	0.407
STOCK.TISSUE.OT.DAY	12		72.844	6.070	0.69	0.762
Residual	142	(2)	1254.027	8.831		
Total	213	(2)	6110.877			

8. Analysis of variance (ANOVA) results of phenolic content in potato tubers of year 1 (2011-2012). Structure used: STOCK*TISSUE*OT*DAY

Table 8.1 Variate: Year 1 – Chlorogenic acid

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
STOCK	2		7355.9	3678.0	4.52	0.013
TISSUE	1		106739.4	106739.4	131.23	<.001
OT	3		1403.6	467.9	0.58	0.632
DAY	2		3049.5	1524.7	1.87	0.157
STOCK.TISSUE	2		63670.2	31835.1	39.14	<.001
STOCK.OT	6		34723.8	5787.3	7.12	<.001
TISSUE.OT	3		38043.4	12681.1	15.59	<.001

STOCK.DAY	4		5444.8	1361.2	1.67	0.160
TISSUE.DAY	2		667.2	333.6	0.41	0.664
OT.DAY	6		25779.0	4296.5	5.28	<.001
STOCK.TISSUE.OT	6		24691.4	4115.2	5.06	<.001
STOCK.TISSUE.DAY	4		9955.8	2488.9	3.06	0.019
STOCK.OT.DAY	12		65249.8	5437.5	6.68	<.001
TISSUE.OT.DAY	6		28327.1	4721.2	5.80	<.001
STOCK.TISSUE.OT.DAY	12		28572.2	2381.0	2.93	0.001
Residual	136	(8)	110620.7	813.4		
Total	207	(8)	504811.2.			

Table 8.2 Variate: Year 1 – Neo-chlorogenic acid

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
STOCK	2		125.4830	62.7415	62.85	<.001
TISSUE	1		120.4462	120.4462	120.65	<.001
OT	3		73.2283	24.4094	24.45	<.001
DAY	2		4.2178	2.1089	2.11	0.125
STOCK.TISSUE	2		99.7264	49.8632	49.95	<.001
STOCK.OT	6		13.9814	2.3302	2.33	0.036
TISSUE.OT	3		44.2980	14.7660	14.79	<.001
STOCK.DAY	4		2.4387	0.6097	0.61	0.656
TISSUE.DAY	2		3.9698	1.9849	1.99	0.141
OT.DAY	6		20.5101	3.4184	3.42	0.004
STOCK.TISSUE.OT	6		31.0533	5.1755	5.18	<.001
STOCK.TISSUE.DAY	4		10.9092	2.7273	2.73	0.032
STOCK.OT.DAY	12		51.9198	4.3267	4.33	<.001
TISSUE.OT.DAY	6		17.3588	2.8931	2.90	0.011
STOCK.TISSUE.OT.DAY	12		54.6952	4.5579	4.57	<.001
Residual	126	(18)	125.7843	0.9983		
Total	197	(18)	662.3266			

Table 8.3 Variate: Year 1 – Crypto-chlorogenic acid

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
STOCK	2		3706.78	1853.39	75.42	<.001
TISSUE	1		8879.73	8879.73	361.35	<.001
OT	3		1318.10	439.37	17.88	<.001
DAY	2		126.02	63.01	2.56	0.081
STOCK.TISSUE	2		8587.23	4293.61	174.72	<.001
STOCK.OT	6		2675.50	445.92	18.15	<.001
TISSUE.OT	3		100.45	33.48	1.36	0.257
STOCK.DAY	4		991.74	247.94	10.09	<.001
TISSUE.DAY	2		466.99	233.49	9.50	<.001
OT.DAY	6		1131.82	188.64	7.68	<.001

STOCK.TISSUE.OT	6		2370.67	395.11	16.08	<.001
STOCK.TISSUE.DAY	4		519.80	129.95	5.29	<.001
STOCK.OT.DAY	12		1264.27	105.36	4.29	<.001
TISSUE.OT.DAY	6		421.01	70.17	2.86	0.012
STOCK.TISSUE.OT.DAY	11	(1)	1665.29	151.39	6.16	<.001
Residual	131	(13)	3219.16	24.57		
Total	201	(14)	31521.90			

Table 8.4 Variate: Year 1 - Caffeoyl-D-glucose

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
STOCK	2		1.81441	0.90720	46.87	<.001
TISSUE	1		0.01850	0.01850	0.96	0.330
OT	3		4.06720	1.35573	70.04	<.001
DAY	2		0.01254	0.00627	0.32	0.724
STOCK.TISSUE	2		1.45640	0.72820	37.62	<.001
STOCK.OT	6		0.26280	0.04380	2.26	0.041
TISSUE.OT	3		2.70931	0.90310	46.66	<.001
STOCK.DAY	4		0.16523	0.04131	2.13	0.080
TISSUE.DAY	2		0.01195	0.00598	0.31	0.735
OT.DAY	6		0.67721	0.11287	5.83	<.001
STOCK.TISSUE.OT	6		0.45361	0.07560	3.91	0.001
STOCK.TISSUE.DAY	4		0.20047	0.05012	2.59	0.039
STOCK.OT.DAY	12		1.14297	0.09525	4.92	<.001
TISSUE.OT.DAY	6		0.26662	0.04444	2.30	0.038
STOCK.TISSUE.OT.DAY	12		0.45111	0.03759	1.94	0.034
Residual	137	(7)	2.65187	0.01936		
Total	208	(7)	15.41723			

Table 8.5 Variate: Year 1 – Feruloylquinic acid

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
STOCK	2		1254.577	627.289	105.19	<.001
TISSUE	1		434.884	434.884	72.92	<.001
OT	3		167.294	55.765	9.35	<.001
DAY	2		253.973	126.987	21.29	<.001
STOCK.TISSUE	2		333.432	166.716	27.96	<.001
STOCK.OT	6		380.333	63.389	10.63	<.001
TISSUE.OT	3		608.272	202.757	34.00	<.001
STOCK.DAY	4		4.447	1.112	0.19	0.945
TISSUE.DAY	2		177.069	88.534	14.85	<.001
OT.DAY	6		137.651	22.942	3.85	0.001
STOCK.TISSUE.OT	6		106.040	17.673	2.96	0.009
STOCK.TISSUE.DAY	4		88.693	22.173	3.72	0.007

STOCK.OT.DAY	12		355.167	29.597	4.96	<.001
TISSUE.OT.DAY	6		59.867	9.978	1.67	0.132
STOCK.TISSUE.OT.DAY	12		208.444	17.370	2.91	0.001
Residual	137	(7)	816.994	5.963		
Total	208	(7)	4846.957			

Table 8.6 Variate: Year 1 - Tyrosine

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
STOCK	2		10.4364	5.2182	26.56	<.001
TISSUE	1		4.1987	4.1987	21.37	<.001
OT	3		9.4693	3.1564	16.06	<.001
DAY	2		0.5710	0.2855	1.45	0.238
STOCK.TISSUE	2		3.6693	1.8346	9.34	<.001
STOCK.OT	6		9.0879	1.5147	7.71	<.001
TISSUE.OT	3		5.1149	1.7050	8.68	<.001
STOCK.DAY	4		1.8439	0.4610	2.35	0.058
TISSUE.DAY	2		1.3259	0.6629	3.37	0.037
OT.DAY	6		1.9709	0.3285	1.67	0.133
STOCK.TISSUE.OT	6		5.4048	0.9008	4.58	<.001
STOCK.TISSUE.DAY	4		0.7149	0.1787	0.91	0.460
STOCK.OT.DAY	12		3.5997	0.3000	1.53	0.122
TISSUE.OT.DAY	6		4.3045	0.7174	3.65	0.002
STOCK.TISSUE.OT.DAY	11	(1)	3.3638	0.3058	1.56	0.119
Residual	133	(11)	26.1341	0.1965		
Total	203	(12)	81.3128			

Table 8.7 Variate: Year 1 - Phenylalanine

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
STOCK	2		1625.66	812.83	39.15	<.001
TISSUE	1		1505.30	1505.30	72.50	<.001
OT	3		2493.84	831.28	40.04	<.001
DAY	2		556.82	278.41	13.41	<.001
STOCK.TISSUE	2		174.64	87.32	4.21	0.017
STOCK.OT	6		590.13	98.36	4.74	<.001
TISSUE.OT	3		608.71	202.90	9.77	<.001
STOCK.DAY	4		553.29	138.32	6.66	<.001
TISSUE.DAY	2		63.96	31.98	1.54	0.218
OT.DAY	6		609.48	101.58	4.89	<.001

STOCK.TISSUE.OT	6		257.60	42.93	2.07	0.061
STOCK.TISSUE.DAY	4		78.93	19.73	0.95	0.437
STOCK.OT.DAY	12		1088.81	90.73	4.37	<.001
TISSUE.OT.DAY	6		92.54	15.42	0.74	0.616
STOCK.TISSUE.OT.DAY	12		783.65	65.30	3.15	<.001
Residual	140	(4)	2906.86	20.76		
Total	211	(4)	13635.21			

Table 8.8 Variate: Year 1 - Tryptophan

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
STOCK	2		107472.	53736.	51.29	<.001
TISSUE	1		13674.	13674.	13.05	<.001
OT	3		38444.	12815.	12.23	<.001
DAY	2		12619.	6309.	6.02	0.003
STOCK.TISSUE	2		4263.	2132.	2.03	0.135
STOCK.OT	6		22224.	3704.	3.54	0.003
TISSUE.OT	3		26285.	8762.	8.36	<.001
STOCK.DAY	4		22225.	5556.	5.30	<.001
TISSUE.DAY	2		5487.	2743.	2.62	0.076
OT.DAY	6		16309.	2718.	2.59	0.020
STOCK.TISSUE.OT	6		8248.	1375.	1.31	0.255
STOCK.TISSUE.DAY	4		17171.	4293.	4.10	0.004
STOCK.OT.DAY	12		33583.	2799.	2.67	0.003
TISSUE.OT.DAY	6		6811.	1135.	1.08	0.375
STOCK.TISSUE.OT.DAY	12		20274.	1689.	1.61	0.094
Residual	143	(1)	149820.	1048.		
Total	214	(1)	496336.			

Appendix C)

1. Analysis of Variance for respiration rate of potato tubers cv. Maris Piper stock 7 (susceptible to BH), stock 12 (susceptible to BH) and stock 3 (non-susceptible to BH) recorded after storage in air at 20^{o}C on sampling days 0 and 7. Potato tubers were initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks. Structure used: STOCK*OT*DAY

Variate: Year 2 – Experiment 1 - CO_2

Source of variation	d.f.	s.s .	m.s.	v.r.	F pr.
STOCK	2	46.015	23.008	22.38	<.001
OT	5	19.098	3.820	3.71	0.037
DAY	1	49.057	49.057	47.71	<.001
STOCK.OT	10	9.010	0.901	0.88	0.581
STOCK.DAY	2	31.978	15.989	15.55	<.001
OT.DAY	5	14.555	2.911	2.83	0.076
Residual	10	10.282	1.028		
Total	35	179.996			

2. Chi-square test results of BH susceptibility between stock 7, stock 12 (susceptible to BH stocks) and stock 3 (non-susceptible to BH) after storage in air at 20^{o}C (Baseline and all outturns included). Tubers were initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks. (P < 0.05).

Table 2.1 Chi-square test results of BH susceptibility between stock 7 and stock 12 (both susceptible to BH).

OBSERVED	BH	NO BH	TOTAL
Stock 7	16	92	108
Stock 12	12	96	108
TOTAL	28	188	216
EXPECTED	BH	NO BH	TOTAL
Stock 7	14	94	108
Stock 12	14	94	108
TOTAL	28	188	216
p= 0.417785907			

Table 2.2 Chi-square test results of BH susceptibility between stock 7 (susceptible to BH) and stock 12 (non-susceptible to BH).

	OBSERVED	BH	NO BH	TOTAL
	Stock 7	16	92	108
	Stock 3	0	108	108
	TOTAL	16	200	216
	EXPECTED	BH	NO BH	TOTAL
	Stock 7	8	100	108
	Stock 3	8	100	108
	TOTAL	16	200	216
p=	< 0.001			

Table 2.3 Chi-square test results of BH susceptibility between stock 12 (susceptible to BH) and stock 12 (non-susceptible to BH).

	OBSERVED	BH	NO BH	TOTAL
	Stock 7	12	96	108
	Stock 3	0	108	108
	TOTAL	12	204	216
	EXPECTED	BH	NO BH	TOTAL
	Stock 7	6	102	108
	Stock 3	6	102	108
	TOTAL	12	204	216
p=	0.0004			

3. Two-sample t-tests for BH incidence and tuber respiration rate, tuber size and tuber weight of potato cv. Maris Piper stock 7 and stock 12 (susceptible to BH stocks) after baseline and storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 4, 8, 12, 16 and 20 weeks. Tubers were initially stored at 1.5^{o}C ($P < 0.05$).

Table 3.1 Two-sample t-test for tuber respiration rate (CO_2) and BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) at baseline.

Summary					
Sample	Size	Standard Mean	Variance	deviation	Standard error of mean
Non-affected	16	6.935	4.358	2.088	0.5219
Affected	2	5.638	0.434	0.659	0.4658

Probability = 0.797

Table 3.2 Two-sample t-test for tuber length (mm) and BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) at baseline.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	97.91	144.9	12.04	3.010
Affected	2	86.13	172.4	13.13	9.285

Probability = 0.893

Table 3.3 Two-sample t-test for tuber diameter (mm) and BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) at baseline.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	72.64	71.98	8.484	2.121
Affected	2	78.42	71.76	8.471	5.990

Probability = 0.188

Table 3.4 Two-sample t-test for tuber weight (g) and BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) at baseline.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	272.9	2686	51.83	12.96
Affected	2	243.9	5898	76.80	54.31

Probability = 0.759

Table 3.5 Two-sample t-test for tuber respiration rate (CO_2) and BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) after storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 4 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	15	5.910	2.388	1.5454	0.3990
Affected	3	5.409	0.146	0.3820	0.2205

Probability = 0.704

Table 3.6 Two-sample t-test for tuber length (mm) and BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 4 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	15	97.93	99.45	9.972	2.575
Affected	3	98.03	70.01	8.367	4.831

Probability = 0.494

Table 3.7 Two-sample t-test for tuber diameter (mm) and BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 4 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	15	78.89	31.34	5.598	1.445
Affected	3	72.04	14.42	3.797	2.192

Probability = 0.969

Table 3.8 Two-sample t-test for tuber weight (g) and BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 4 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	15	262.0	1995	44.66	11.53
Affected	3	232.0	2864	53.51	30.90

Probability = 0.842

Table 3.9 Two-sample t-test for tuber respiration rate (CO_2) and BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 12 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	4.725	0.5739	0.7576	0.1894
Affected	2	4.558	0.0016	0.0405	0.0286

Probability = 0.617

Table 3.10 Two-sample t-test for tuber length (mm) with BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 12 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	97.38	95.34	9.764	2.441
Affected	2	92.62	29.26	5.409	3.825

Probability = 0.742

Table 3.11 Two-sample t-test for tuber diameter (mm) with BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 12 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	78.12	10.937	3.307	0.827
Affected	2	75.11	6.125	2.475	1.750

Probability = 0.882

Table 3.12 Two-sample t-test for tuber weight (g) with BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 12 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	263.1	1880.3	43.36	10.841
Affected	2	248.5	40.5	6.36	4.500

Probability = 0.675

Table 3.13 Two-sample t-test for tuber respiration rate (CO_2) and BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 16 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	14	5.074	0.8854	0.9410	0.2515
Affected	4	4.286	0.4497	0.6706	0.3353

Probability = 0.930

Table 3.14 Two-sample t-test for tuber length (mm) with BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 16 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	14	96.40	171.6	13.10	3.501
Affected	4	97.05	86.9	9.32	4.661

Probability = 0.464

Table 3.15 Two-sample t-test for tuber diameter (mm) with BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 16 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	14	74.27	16.25	4.031	1.077
Affected	4	77.21	14.93	3.863	1.932

Probability = 0.106

Table 3.16 Two-sample t-test for tuber weight (g) with BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 16 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	14	242.5	1517	38.95	10.41
Affected	4	261.0	2122	46.06	23.03

Probability = 0.216

Table 3.17 Two-sample t-test for tuber respiration rate (CO_2) and BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 20 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	14	4.560	0.5370	0.7328	0.1958
Affected	4	3.919	0.1830	0.4277	0.2139

Probability = 0.941

Table 3.18 Two-sample t-test for tuber length (mm) with BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 20 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	14	89.88	75.93	8.714	2.329
Affected	4	90.30	19.46	4.411	2.206

Probability = 0.464

Table 3.19 Two-sample t-test for tuber diameter (mm) with BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 16 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	14	74.75	19.24	4.386	1.172
Affected	4	76.53	6.27	2.505	1.252

Probability = 0.228

Table 3.20 Two-sample t-test for tuber weight (g) with BH incidence of potato cv. Maris Piper stock 7 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 20 weeks.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	14	229.9	1600	40.00	10.69
Affected	4	239.2	1188	34.47	17.24

Probability = 0.340

4. Two-sample t-test results for BH incidence and tuber length, diameter and weight of potato cv. Maris Piper stock 12 (susceptible to BH) after baseline and storage in air at 20°C. Tubers were initially stored at 1.5°C for 4, 8, 12, 16 and 20 weeks ($P < 0.05$).

Table 4.1 Two-sample t-test for tuber respiration rate (CO_2) and BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) at baseline.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	6.675	5.867	2.422	0.6055
Affected	2	4.275	0.010	0.101	0.0713

Probability = 0.904

Table 4.2 Two-sample t-test for tuber length (mm) with BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) at baseline.

Summary					
Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	104.9	73.57	8.577	2.144
Affected	2	97.8	0.13	0.361	0.255

Probability = 0.863

Table 4.3 Two-sample t-test for tuber diameter (mm) with BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) at baseline.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	75.70	10.044	3.169	0.792
Affected	2	72.49	4.836	2.199	1.555

Probability = 0.906

Table 4.4 Two-sample t-test for tuber weight (g) with BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) at baseline.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	272.4	1104.8	33.24	8.310
Affected	2	272.0	102.4	10.12	7.155

Probability = 0.507

Table 4.5 Two-sample t-test for tuber respiration rate (CO_2) and BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) after storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 4 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	6.297	3.623	1.903	0.4759
Affected	2	4.272	0.369	0.607	0.4294

Probability = 0.918

Table 4.6 Two-sample t-test for tuber length (mm) with BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) after storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 4 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	105.3	220.8	14.861	3.715
Affected	2	104.9	0.6	0.785	0.555

Probability = 0.516

Table 4.7 Two-sample t-test for tuber diameter (mm) with BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) after storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 4 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	76.82	18.77	4.332	1.083
Affected	2	80.31	6.52	2.553	1.805

Probability = 0.144

Table 4.8 Two-sample t-test for tuber weight (g) with BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) after storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 4 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	287.3	4048	63.63	15.91
Affected	2	306.6	119	10.89	7.70

Probability = 0.341

Table 4.9 Two-sample t-test for tuber respiration rate (CO_2) and BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) after storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 12 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	14	4.279	1.209	1.100	0.2939
Affected	4	5.162	1.405	1.185	0.5926

Probability = 0.091

Table 4.10 Two-sample t-test for tuber length (mm) with BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 12 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	14	104.4	240.9	15.52	4.148
Affected	4	98.7	37.4	6.12	3.059

Probability = 0.758

Table 4.11 Two-sample t-test for tuber diameter (mm) with BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 12 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	14	74.72	6.19	2.489	0.665
Affected	4	77.15	32.39	5.691	2.846

Probability = 0.230

Table 4.12 Two-sample t-test for tuber weight (g) with BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 12 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	14	273.3	2326	48.23	12.89
Affected	4	269.9	1276	35.73	17.86

Probability = 0.551

Table 4.13 Two-sample t-test for tuber respiration rate (CO_2) and BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 16 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	4.222	0.7372	0.8586	0.2146
Affected	2	4.176	0.0008	0.0286	0.0202

Probability = 0.529

Table 4.14 Two-sample t-test for tuber length (mm) with BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) after storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 16 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	104.8	107.09	10.348	2.587
Affected	2	117.5	53.56	7.319	5.175

Probability = 0.058

Table 4.15 Two-sample t-test for tuber diameter (mm) with BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) after storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 16 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	76.80	20.84	4.565	1.1412
Affected	2	77.57	0.55	0.742	0.5250

Probability = 0.409

Table 4.16 Two-sample t-test for tuber weight (g) with BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) after storage in air at 20^{o}C. Tubers were initially stored at 1.5^{o}C for 16 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	281.6	1334.4	36.53	9.13
Affected	2	356.2	283.2	16.83	11.90

Probability = 0.007

Table 4.17 Two-sample t-test for tuber respiration rate (CO_2) and BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 20 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	4.758	1.2178	1.1035	0.2759
Affected	2	4.826	0.7257	0.8519	0.6024

Probability = 0.468

Table 4.18 Two-sample t-test for tuber length (mm) with BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 20 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	97.26	75.17	8.670	2.168
Affected	2	100.42	7.03	2.652	1.875

Probability = 0.312

Table 4.19 Two-sample t-test for tuber diameter (mm) with BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 20 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	75.56	18.037	4.247	1.0617
Affected	2	73.53	0.000	0.014	0.0100

Probability = 0.962

Table 4.20 Two-sample t-test for tuber weight (g) with BH incidence of potato cv. Maris Piper stock 12 (susceptible to BH) after storage in air at 20°C. Tubers were initially stored at 1.5°C for 20 weeks.

Summary

Sample	Size	Mean	Variance	Standard deviation	Standard error of mean
Non-affected	16	261.9	1753.3	41.87	10.47
Affected	2	251.9	214.2	14.64	10.35

Probability = 0.626

Table 4.21 Correlation matrix comparing the tuber length, diameter and weight with the BH incidence for potato cv. Maris Piper stock 12 after storage in air at 20°C. Tubers were initially stored at 1.5°C for 16 weeks.

BH	1	-				
Weight	2	0.5723	-			
Diameter	3	0.0581	0.2314	-		
Length	4	0.3838	0.7699	-0.1786	-	
CO_2	5	-0.0187	0.4362	-0.2574	0.5320	-
		1	2	3	4	5

Two-sided test of correlations different from zero

BH	1	-				
Weight	2	0.0131	-			
Diameter	3	0.8190	0.3555	-		
Length	4	0.1158	<0.001	0.4782	-	
CO_2	5	0.9414	0.0704	0.3024	0.0231	-
		1	2	3	4	5

5. Analysis of variance (ANOVA) results of sugar content in potato tubers of experiment 1 in year 2 (2012-2013). Structure used: STOCK*TISSUE*OT

Table 5.1 Variate: Year 2 – experiment 1 – Fructose

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TISSUE	1	16209.5	16209.5	84.58	<.001
OT	5	5974.6	1194.9	6.23	<.001
TISSUE.OT	5	1125.6	225.1	1.17	0.335
OT.BH_L	17	3929.5	231.1	1.21	0.294
TISSUE.OT.BH_L	17	1717.2	101.0	0.53	0.926
Residual	50	9582.8	191.7		
Total	95	38539.2			

Table 5.2 Variate: Year 2 – experiment 1 – Fructose log_10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TISSUE	1	1.30913	1.30913	92.42	<.001
OT	5	0.85390	0.17078	12.06	<.001
TISSUE.OT	5	0.05021	0.01004	0.71	0.620
OT.BH_L	17	0.71009	0.04177	2.95	0.002

TISSUE.OT.BH_L	17	0.16965	0.00998	0.70	0.783
Residual	50	0.70823	0.01416		
Total	95	3.80120			

Table 5.3 Variate: Year 2 – experiment 1 – Glucose

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TISSUE	1	17116.5	17116.5	67.60	<.001
OT	5	3748.2	749.6	2.96	0.020
TISSUE.OT	5	918.2	183.6	0.73	0.608
OT.BH_L	17	8636.3	508.0	2.01	0.029
TISSUE.OT.BH_L	17	2927.8	172.2	0.68	0.807
Residual	50	12660.0	253.2		
Total	95	46007.1			

Table 5.4 Variate: Year 2 – experiment 1 – Glucose log_10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TISSUE	1	1.46223	1.46223	64.77	<.001
OT	5	0.46090	0.09218	4.08	0.003
TISSUE.OT	5	0.06729	0.01346	0.60	0.703
OT.BH_L	17	1.18471	0.06969	3.09	<.001
TISSUE.OT.BH_L	17	0.25447	0.01497	0.66	0.822
Residual	50	1.12879	0.02258		
Total	95	4.55839			

Table 5.5 Variate: Year 2 – experiment 1 – Sucrose

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
TISSUE	1		19.90	19.90	0.31	0.578
OT	5		3359.90	671.98	10.60	<.001
TISSUE.OT	5		35.60	7.12	0.11	0.989
OT.BH_L	17		6062.24	356.60	5.62	<.001
TISSUE.OT.BH_L	17		757.39	44.55	0.70	0.784
Residual	45	(5)	2854.07	63.42		
Total	90	(5)	11635.29			

Table 5.6 Variate Year 2 – experiment 1 – Sucrose log_10

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
TISSUE	1		0.01314	0.01314	1.11	0.297
OT	5		0.62310	0.12462	10.56	<.001
TISSUE.OT	5		0.00520	0.00104	0.09	0.994
OT.BH_L	17		1.58989	0.09352	7.92	<.001
TISSUE.OT.BH_L	17		0.14445	0.00850	0.72	0.767
Residual	45	(5)	0.53118	0.01180		
Total	90	(5)	2.68189			

6. Analysis of variance (ANOVA) results of phenolic content in potato tubers of experiment 1 in year 2 (2012-2013). Structure used: STOCK*TISSUE*OT.

Table 6.1 Variate: Year 2 – experiment 1 - Chlorogenic acid

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
TISSUE	1		101823.	101823.	1.05	0.311
OT	5		1499292.	299858.	3.09	0.017
TISSUE.OT	5		171135.	34227.	0.35	0.878
OT.BH_L	16	(1)	2218123.	138633.	1.43	0.168
TISSUE.OT.BH_L	16	(1)	1367669.	85479.	0.88	0.593
Residual	49	(1)	4755641.	97054.		
Total	92	(3)	10080403.			

Table 6.2 Variate: Year 2 – experiment 1 - Chlorogenic acid log_10

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
TISSUE	1		0.02512	0.02512	0.33	0.569
OT	5		1.51002	0.30200	3.95	0.004
TISSUE.OT	5		0.07537	0.01507	0.20	0.962
OT.BH_L	16	(1)	2.87750	0.17984	2.35	0.011
TISSUE.OT.BH_L	16	(1)	1.44983	0.09061	1.19	0.312
Residual	49	(1)	3.74429	0.07641		
Total	92	(3)	9.66647			

Table 6.3 Variate: Year 2 – experiment 1 – Neo-chlorogenic acid

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
TISSUE	1		236.959	236.959	43.45	<.001
OT	5		62.502	12.500	2.29	0.064
TISSUE.OT	5		28.296	5.659	1.04	0.409
OT.BH_L	15	(2)	187.779	12.519	2.30	0.019
TISSUE.OT.BH_L	13	(4)	160.828	12.371	2.27	0.024
Residual	39	(11)	212.697	5.454		
Total	78	(17)	776.040			

Table 6.4 Variate: Year 2 – experiment 1 – Neo-chlorogenic acid log_10

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
TISSUE	1		6.39365	6.39365	168.32	<.001
OT	5		2.04996	0.40999	10.79	<.001
TISSUE.OT	5		0.52741	0.10548	2.78	0.031
OT.BH_L	15	(2)	4.32747	0.28850	7.59	<.001
TISSUE.OT.BH_L	13	(4)	3.35069	0.25775	6.79	<.001
Residual	38	(12)	1.44346	0.03799		
Total	77	(18)	13.02268			

Table 6.5 Variate: Year 2 – experiment 1 – Crypto-Chlorogenic acid

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
TISSUE	1		2273.5	2273.5	14.09	<.001
OT	5		3356.8	671.4	4.16	0.003
TISSUE.OT	5		902.7	180.5	1.12	0.364
OT.BH_L	17		6016.4	353.9	2.19	0.018
TISSUE.OT.BH_L	17		8334.2	490.2	3.04	0.001
Residual	45	(5)	7259.9	161.3		
Total	90	(5)	26732.3			

Table 6.6 Variate: Year 2 – experiment 1 – Crypto-Chlorogenic acid log_10

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
TISSUE	1		0.47095	0.47095	15.98	<.001
OT	5		0.93182	0.18636	6.32	<.001
TISSUE.OT	5		0.18823	0.03765	1.28	0.290
OT.BH_L	17		2.71715	0.15983	5.42	<.001
TISSUE.OT.BH_L	17		2.50154	0.14715	4.99	<.001

Residual	45	(5)	1.32653	0.02948	
Total	90	(5)	7.31328		

Table 6.7 Variate: Year 2 – experiment 1 – Quercetin-3,4-O-diglucoside

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
TISSUE	1		0.102	0.102	0.01	0.913
OT	5		140.958	28.192	3.35	0.012
TISSUE.OT	5		6.922	1.384	0.16	0.974
OT.BH_L	15	(2)	806.408	53.761	6.38	<.001
TISSUE.OT.BH_L	13	(4)	13.198	1.015	0.12	1.000
Residual	46	(4)	387.428	8.422		
Total	85	(10)	1326.261			

Table 6.8 Variate: Year 2 – experiment 1 – Quercetin-3,4-O-diglucoside log_10

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
TISSUE	1		0.12489	0.12489	1.35	0.251
OT	5		1.42529	0.28506	3.08	0.018
TISSUE.OT	5		0.25785	0.05157	0.56	0.732
OT.BH_L	15	(2)	10.02020	0.66801	7.23	<.001
TISSUE.OT.BH_L	13	(4)	0.44696	0.03438	0.37	0.972
Residual	46	(4)	4.25166	0.09243		
Total	85	(10)	15.02563			

Table 6.9 Variate: Year 2 – experiment 1 - Rutin

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
TISSUE	1		1236.0	1236.0	2.00	0.164
OT	5		9395.5	1879.1	3.05	0.018
TISSUE.OT	5		679.2	135.8	0.22	0.952
OT.BH_L	16	(1)	57423.6	3589.0	5.82	<.001
TISSUE.OT.BH_L	14	(3)	3620.8	258.6	0.42	0.961
Residual	47	(3)	28991.7	616.8		
Total	88	(7)	98900.3			

Table 6.10 Variate: Year 2 – experiment 1 – Rutin log_10

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
TISSUE	1		0.3299	0.3299	3.23	0.079
OT	5		4.3224	0.8645	8.47	<.001
TISSUE.OT	5		0.0879	0.0176	0.17	0.972
OT.BH_L	16	(1)	11.6067	0.7254	7.11	<.001
TISSUE.OT.BH_L	14	(3)	0.8212	0.0587	0.57	0.871
Residual	47	(3)	4.7966	0.1021		
Total	88	(7)	19.1142			

Table 6.11 Variate: Year 2 – experiment 1 – Phenylalanine

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
TISSUE	1		127.40	127.40	3.72	0.060
OT	5		567.96	113.59	3.31	0.012
TISSUE.OT	5		355.20	71.04	2.07	0.085
OT.BH_L	17		1245.54	73.27	2.14	0.020
TISSUE.OT.BH_L	17		527.51	31.03	0.91	0.572
Residual	49	(1)	1679.24	34.27		
Total	94	(1)	4493.61			

Table 6.12 Variate: Year 2 – experiment 1 – Phenylalanine log_10

Source of variation	d.f.	(m.v.)	s.s.	m.s.	v.r.	F pr.
TISSUE	1		0.13269	0.13269	6.19	0.016
OT	5		0.34872	0.06974	3.25	0.013
TISSUE.OT	5		0.20880	0.04176	1.95	0.103
OT.BH_L	17		1.00885	0.05934	2.77	0.003
TISSUE.OT.BH_L	17		0.49950	0.02938	1.37	0.192
Residual	49	(1)	1.05049	0.02144		
Total	94	(1)	3.24511			

Table 6.13 Variate: Year 2 – experiment 1 – Tryptophan

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TISSUE	1	2713.	2713.	0.89	0.351
OT	5	34996.	6999.	2.29	0.060
TISSUE.OT	5	6790.	1358.	0.44	0.815
OT.BH_L	17	58960.	3468.	1.14	0.350
TISSUE.OT.BH_L	17	34943.	2055.	0.67	0.813
Residual	50	152744.	3055.		

Total	95	291145.			

Table 6.14 Variate: Year 2 – experiment 1 – Tryptophan_log10

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
TISSUE	1	0.06907	0.06907	1.37	0.247
OT	5	0.69019	0.13804	2.74	0.029
TISSUE.OT	5	0.09400	0.01880	0.37	0.864
OT.BH_L	17	0.81297	0.04782	0.95	0.524
TISSUE.OT.BH_L	17	0.57095	0.03359	0.67	0.818
Residual	50	2.51542	0.05031		
Total	95	4.75261			

7. Analysis of variance for respiration rate of potato tubers cv. Maris Piper stock 7 (susceptible to BH), stock 12 (susceptible to BH) and stock 3 (non-susceptible to BH) recorded after storage at 20^{o}C in 4 gas combinations (*viz.* A = 21% O_2, B = 10% CO_2, C = 10% O_2 and D = 5% O_2) on sampling days 3, 7, 10 and 14. Baseline storage in air only (day 0). Structure used: Stock*CACODE/(CA*DAY)

Variate: Year 2 – Experiment 2 - CO_2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
REPS stratum	8	162.928	20.366	2.53	
REPS.*Units* stratum					
STOCK	2	114.629	57.314	7.13	<.001
CACODE	1	0.301	0.301	0.04	0.847
STOCK.CACODE	2	111.508	55.754	6.93	0.001
CACODE.CA	3	3497.304	1165.768	144.99	<.001
CACODE.DAY	3	757.294	252.431	31.40	<.001
STOCK.CACODE.CA	6	440.856	73.476	9.14	<.001
STOCK.CACODE.DAY	6	50.192	8.365	1.04	0.398
CACODE.CA.DAY	9	685.643	76.183	9.48	<.001
STOCK.CACODE.CA.DAY	18	122.150	6.786	0.84	0.648
Residual	399	3208.054	8.040		
Total	457	9108.646			

8. Generalized Linear Model results for BH incidence of potato tubers cv. Maris Piper stock 7 (susceptible to BH) after storage at 20°C in 4 gas combinations (*viz.* A = 21% O_2, B = 10% CO_2, C = 10% O_2 and D = 5% O_2) on sampling days 3, 7, 10 and 14.

Summary of analysis

Source	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	6	10.939	1.823	1.82	0.090
Residual	9	9.748	1.083		
Total	15	20.687	1.379		

Dispersion parameter is fixed at 1.00.

Estimates of parameters

Parameter	estimate	s.e.	t(*)	t pr.	antilog of estimate
Constant	-3.24	1.11	-2.92	0.003	0.03932
Day 7	-0.343	0.833	-0.41	0.681	0.7097
Day 10	0.000	0.782	0.00	1.000	1.0000
Day 14	-1.55	1.17	-1.33	0.184	0.2126
CA B	1.17	1.18	0.99	0.323	3.219
CA C	0.00	1.44	0.00	1.000	1.0000
CA D	2.17	1.10	1.97	0.049	8.770

9. Generalized Linear Model results for BH incidence of potato tubers cv. Maris Piper stock 7 (susceptible to BH) after storage at 20°C in 4 gas combinations (*viz.* A = 21% O_2, B = 10% CO_2, C = 10% O_2 and D = 5% O_2) on sampling days 3, 7, 10 and 14.

Summary of analysis

Source	d.f.	deviance	mean deviance	deviance ratio	approx chi pr
Regression	6	12.893	2.1489	2.15	0.045
Residual	9	7.353	0.8170		
Total	15	20.247	1.3498		

Dispersion parameter is fixed at 1.00.

Estimates of parameters

Parameter	estimate	s.e.	t(*)	t pr.	antilog of estimate
Constant	-13.2	61.7	-0.21	0.831	1.935E-06
Day 7	-0.73	1.26	-0.58	0.560	0.4803
Day 10	1.277	0.870	1.47	0.142	3.586
Day 14	-0.73	1.26	-0.58	0.560	0.4803

CA B	10.5	61.7	0.17	0.865	36451.
CA C	10.5	61.7	0.17	0.865	36451.
CA D	10.8	61.7	0.18	0.860	51379.

Appendix D)

1. Total ion chromatograms of flesh and heart samples of stock 23 (susceptible to BH) with tissue discoloration and control (e) in negative and positive mode.

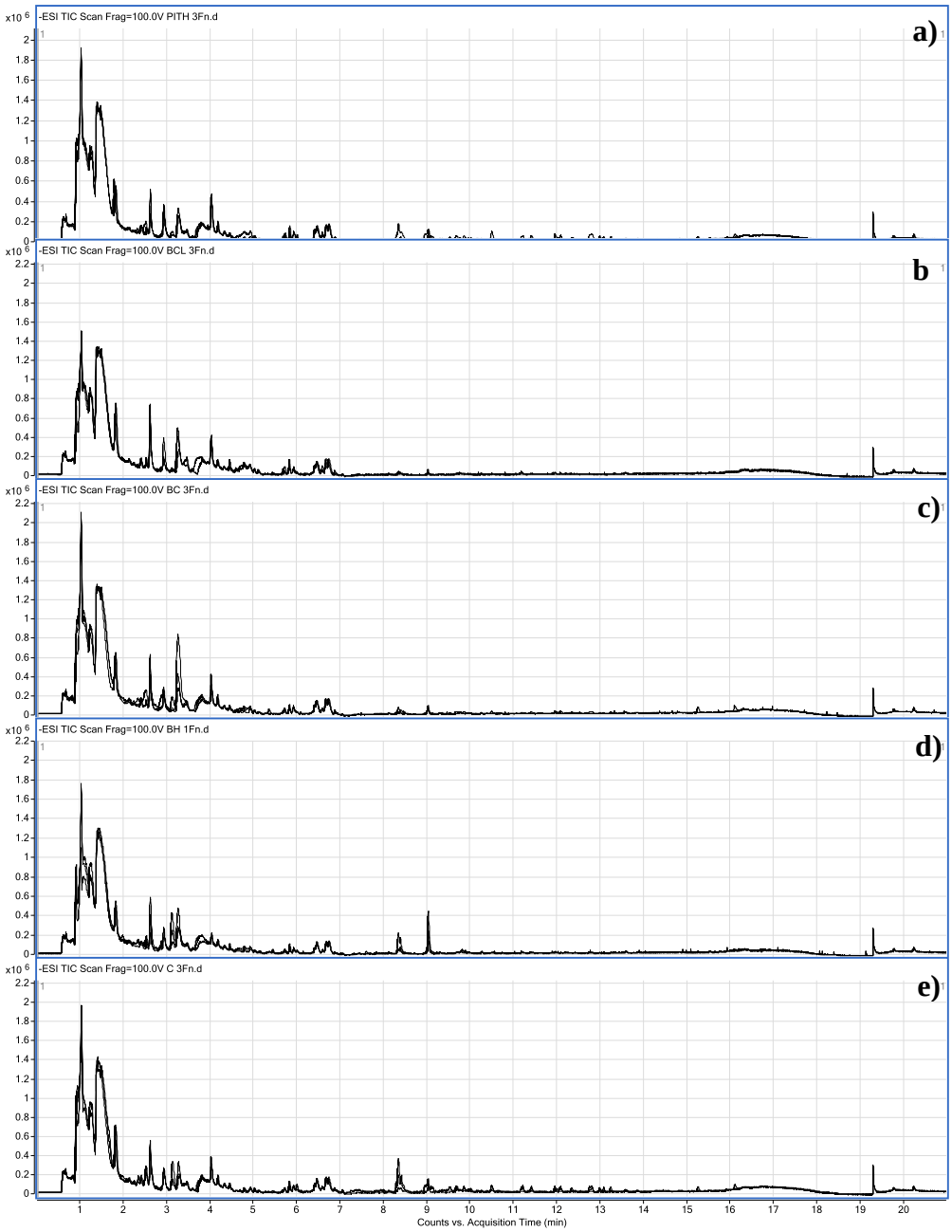

Figure 1.1 Total ion chromatograms of flesh samples of stock 23 (susceptible to BH) with pith (a), BCL (b), BC (c) and BH (d) discoloration and control (e) in negative mode.

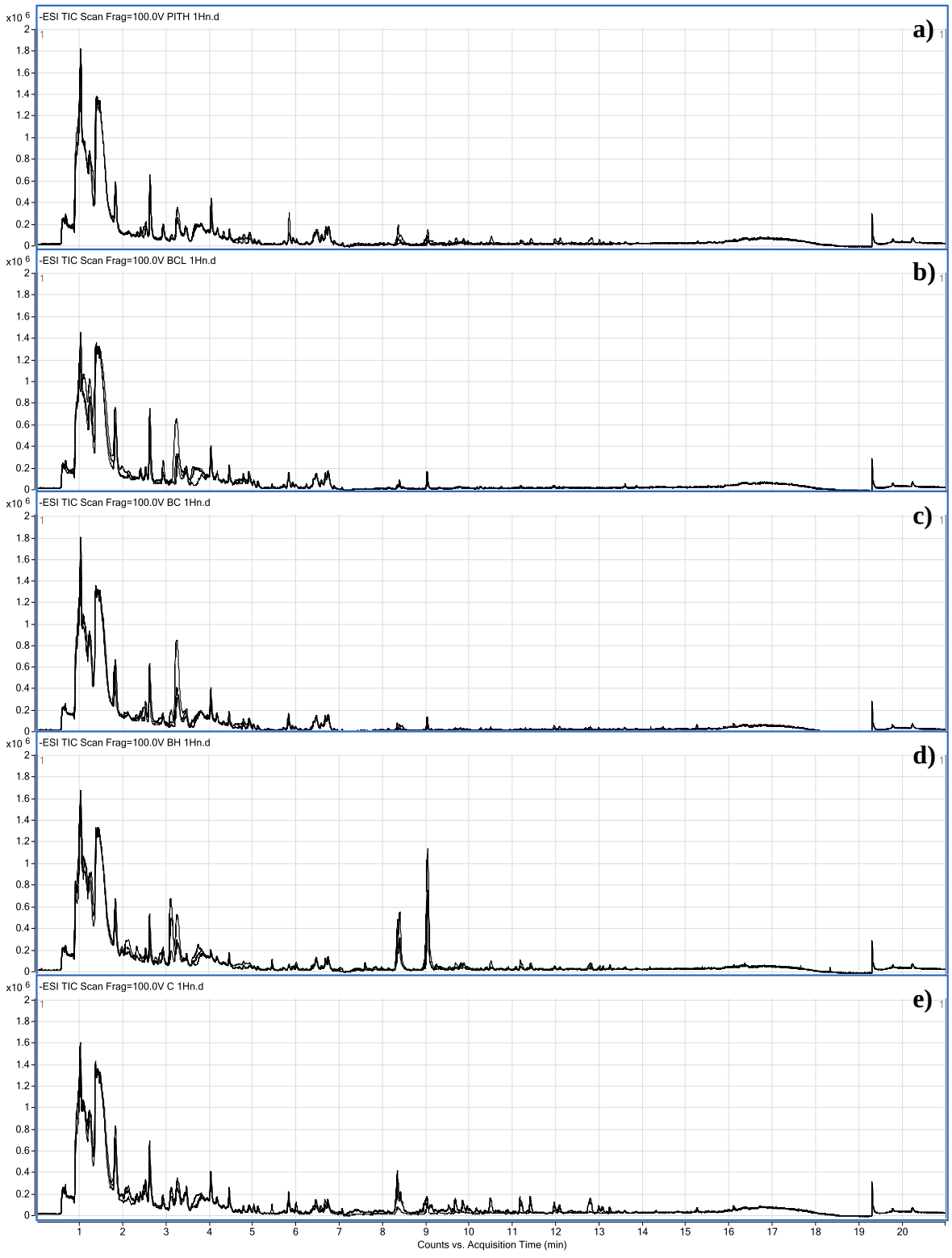

Figure 1.2 Total ion chromatograms of heart samples of stock 23 (susceptible to BH) with pith (a), BCL (b), BC (c) and BH (d) discoloration and control (e) in negative mode.

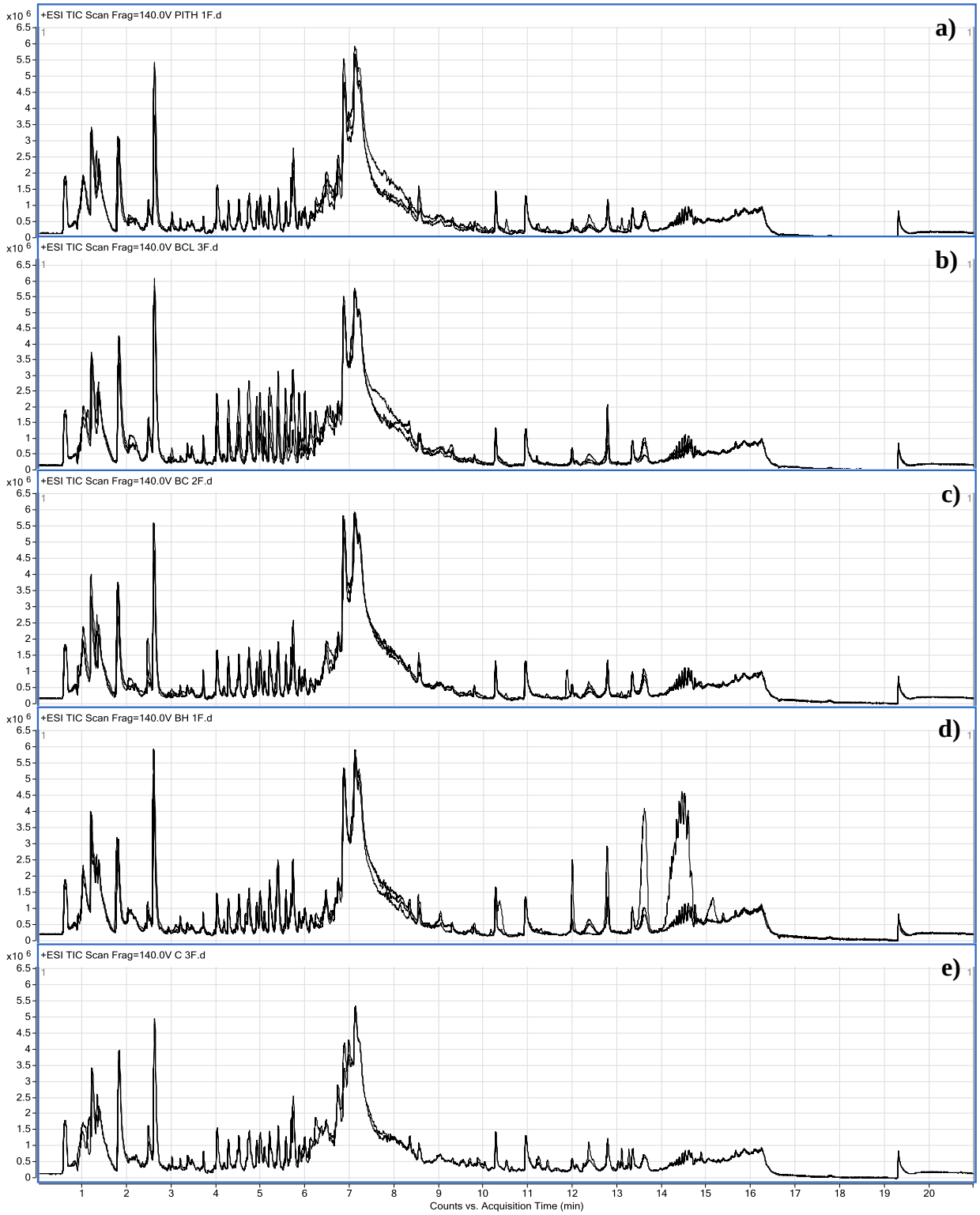

Figure 1.3 Total ion chromatograms of flesh samples of stock 23 (susceptible to BH) with pith (a), BCL (b), BC (c) and BH (d) discoloration and control (e) in positive mode.

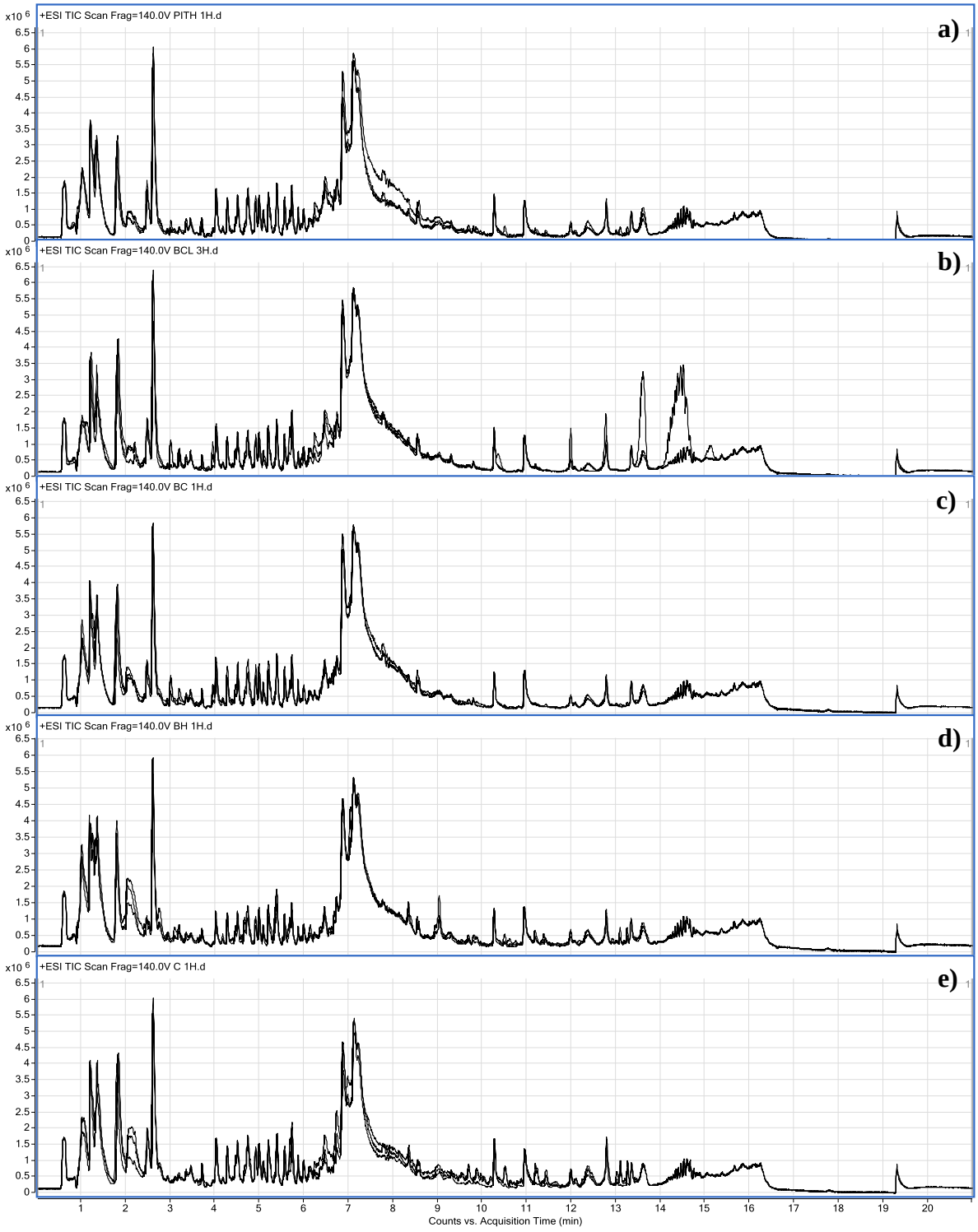

Figure 1.4 Total ion chromatograms of heart samples of stock 23 (susceptible to BH) with pith (a), BCL (b), BC (c) and BH (d) discoloration and control (e) in positive mode.

2. Total ion chromatograms of flesh and heart control samples of stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) in negative and positive mode.

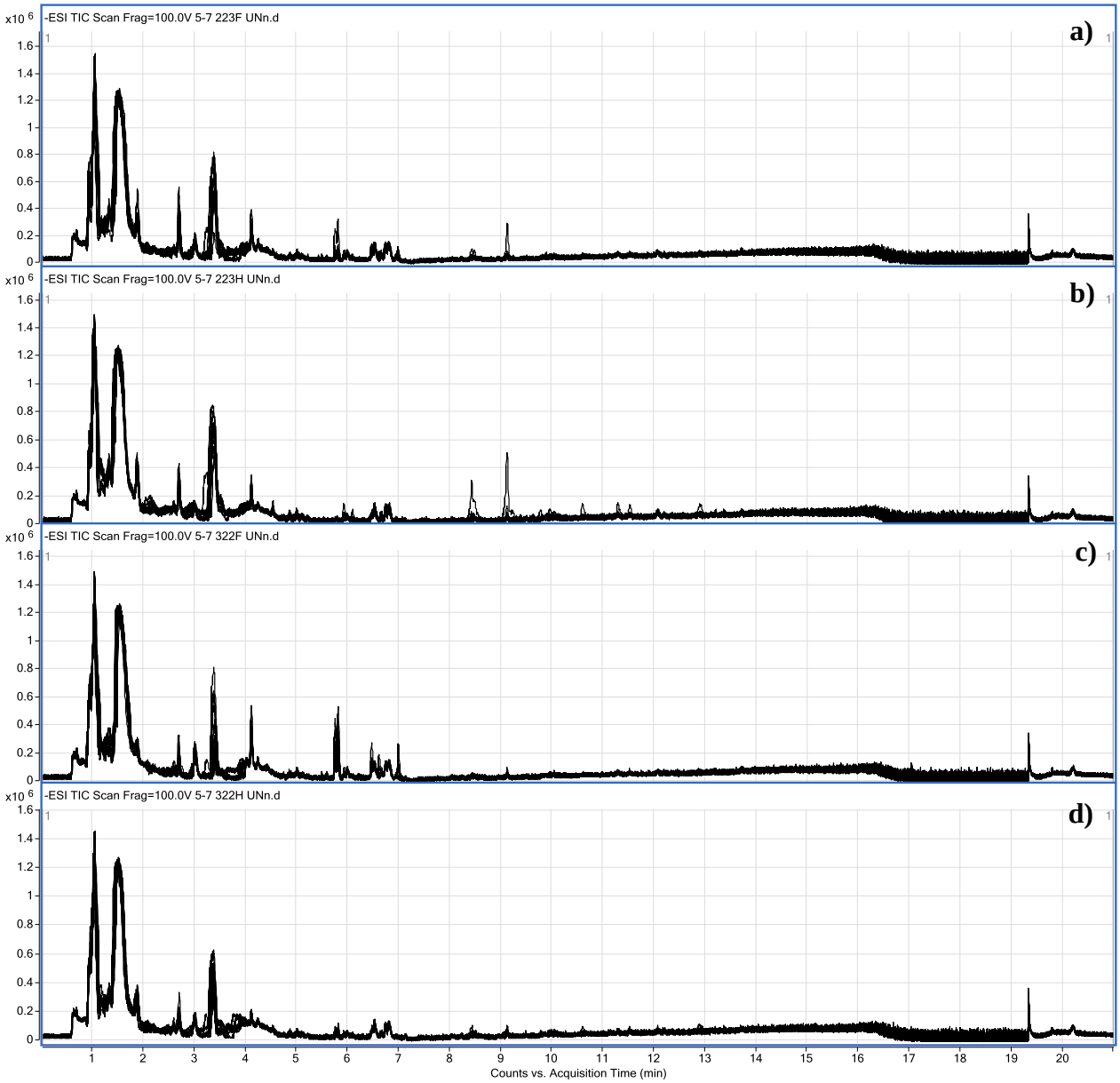

Figure 2.1 Total ion chromatograms of flesh and heart control samples of stock 23 (susceptible to BH) (a, b) and stock 12 (non-susceptible to BH) (c, d) in negative mode.

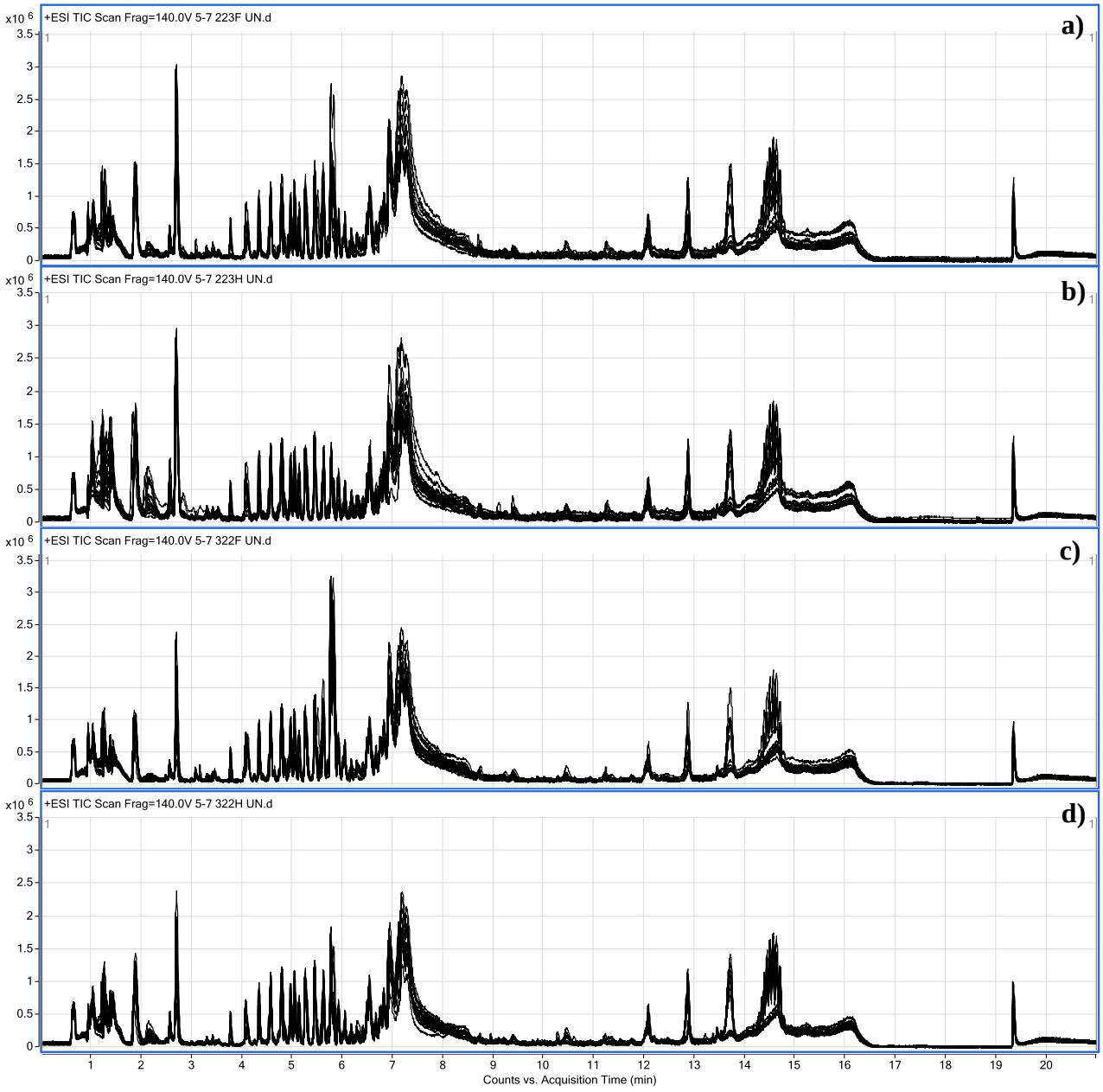

Figure 2.2 Total ion chromatograms of flesh and heart control samples of stock 23 (susceptible to BH) (a, b) and stock 12 (non-susceptible to BH) (c, d) in positive mode.

2. Metabolomic differences between affected and non-affected potato tuber tissues (negative mode).

Table 2.1 Analysis of Variance (ANOVA) results of 'discoloration only' interpretation of in negative mode ('Experiment A') ($P < 0.05$).

Compound	*p*	*p* (Corr)	Formula	Composite Spectrum
273.9659@0.636	0.0214	0.0337		(272.95825, 63577.73)(273.9596, 16927.633)(274.9567, 16492.197)(275.9582, 2381.3865)(276.95575, 712.45996)
7,9-Dimethyluric acid	0.0052	0.0103	C7 H8 N4 O3	(195.05287, 82119.03)(196.0563, 4263.237)(197.05734, 440.69666)
2-Methylcitric acid	1.10E-05	1.10E-04	C7 H10 O7	(411.07706, 1144.1749)(205.03525, 14488.662)(206.0386, 1155.4001)
C21 H28 N8 O17	0.004	0.009	C21 H28 N8 O17	(663.15076, 24864.809)(664.15326, 5378.492)(665.1545, 1303.7916)
2-Amino-5-formylamino-6-(5-phospho-D-ribosylamino)pyrimidin-4(3H)-one	0.004	0.009	C10 H16 N5 O9 P	(761.12756, 5095.12)(762.1299, 1151.38)(380.06042, 15469.983)(381.06302, 1905.9751)
Guanosine 3'-phosphate	9.71E-04	0.003	C10 H14 N5 O8 P	(725.1063, 1270.1124)(726.1094, 559.1)(362.0502, 22461.398)(363.05295, 2656.1365)(364.05484, 663.07996)
Methylisocitric acid	4.21E-04	0.002	C7 H10 O7	(205.03491, 48789.254)(206.03839, 3224.3274)(207.04034, 498.02795)
Pseudouridine 5'-phosphate	2.00E-04	0.001	C9 H13 N2 O9 P	(647.06256, 1771.7222)(648.0651, 926.0)(323.028, 40346.03)(324.03125, 3941.0098)(325.03256, 842.5667)
2',3'-Cyclic UMP	0.004	0.009	C9 H11 N2 O8 P	(611.0418, 2377.9998)(612.04486, 832.10004)(305.0175, 37847.59)(306.02066, 3428.1833)(307.02246, 691.5751)
Molybdopterin precursor Z	0.004	0.009	C10 H12 N5 O7 P	(689.08514, 2331.6816)(690.0879, 829.45996)(344.03958, 37518.56)(345.04245, 4088.4414)(346.04456, 716.07)
5-Acetamidovalerate	9.54E-04	0.003	C7 H13 N O3	(158.08218, 14966.922)(159.08543, 1269.7356)
C16 H20 O13 S	1.17E-09	3.51E-08	C16 H20 O13 S	(451.05457, 119400.555)(452.05783, 22067.7)(453.05862, 5822.2764)(454.05997, 86.62593)
Glu Ala Trp	9.16E-09	1.37E-07	C19 H24 N4 O6	(807.32623, 578.0)(403.1607, 14756.6875)(404.16388, 2822.1719)(405.16583, 713.1375)
1510.2971@6.457234	0.010	0.016		(1509.2883, 23517.22)(1510.2896, 12031.989)(1511.2897, 1777.9436)
1435.2827@6.733067	0.006	0.010		(1434.2748, 25363.764)(1435.2755, 6807.0137)(1436.2745, 878.6518)
1434.9487@6.733134	0.006	0.011		(1433.9407, 26085.094)(1434.942, 12161.876)(1435.9419, 1721.7366)
9S,10S,11R-trihydroxy-12Z-octadecenoic acid	8.82E-04	0.003	C18 H34 O5	(659.4713, 1245.95)(329.23288, 43508.137)(330.23633, 4656.7456)(331.2387, 863.55005)

12,13-dihydroxy-11-methoxy-9-octadecenoic acid	0.004	0.009	C19 H36 O5	(343.24857, 29326.818)(344.25186, 5444.0503)(345.2543, 1107.0857)
C7 H4 N4 O2	7.51E-05	5.63E-04	C7 H4 N4 O2	(175.0264, 18534.426)(176.0298, 1262.8212)(177.04199, 986.2334)

Table 2.2 Fold change analysis results of 'discoloration only' interpretation in negative mode ('Experiment A').

Compound	Replication					Relative abundance normalized				
	BC (6)	BCL (6)	BH (6)	control (6)	PITH (6)	BC (6)	BCL (6)	BH (6)	control (6)	PITH (6)
2-Methylcitric acid	4	6	0	6	5	10.118	15.521	0	15.773	13.005
C21 H28 N8 O17	2	0	3	6	2	5.323	0	7.354	15.772	5.219
2-Amino-5-formylamino-6-(5-phospho-D-ribosylamino)pyrimidin-4(3H)-one	2	0	2	6	2	5.254	0	5.118	15.856	5.281
Guanosine 3'-phosphate	2	0	1	6	2	5.694	0	2.704	17.019	5.473
Pseudouridine 5'-phosphate	1	0	1	6	2	2.972	0	2.818	18.091	5.771
2',3'-Cyclic UMP	2	0	2	6	2	6.052	0	5.641	17.808	5.853
Molybdopterin precursor Z	2	0	2	6	2	5.861	0	5.459	17.276	5.678
5-Acetamidovalerate	3	6	0	4	1	7.662	15.409	0	10.470	2.513
C16 H20 O13 S	6	6	6	6	6	18.686	18.987	17.229	18.827	19.100
Glu Ala Trp	6	6	1	6	6	15.283	15.262	2.472	15.523	15.328
9S,10S,11R-trihydroxy-12Z-octadecenoic acid	2	0	1	6	3	5.282	0	2.956	17.599	8.384
12,13-dihydroxy-11-methoxy-9-octadecenoic acid	2	0	3	6	3	5.247	0	8.123	17.087	8.304
C7 H4 N4 O2	6	4	0	3	6	15.897	10.176	0	7.624	15.401

Table 2.3 Analysis of Variance (ANOVA) results of 'tissue vs. discoloration' interpreting BH against control in negative mode ('Experiment A') ($P < 0.05$).

Compound	*p*	*p* (Corr)	Formula	Composite spectrum
C8 H7 N3 O S	8.31E-15	2.74E-13	C8 H7 N3 O S	(192.02261, 87489.34)(193.02377, 17575.148)(194.02686, 282.59164)
135.9019@1.8312	7.72E-04	0.006370279		(134.89459, 13836.8125)(135.8948, 1913.7534)(136.89172, 5401.8667)(137.89177, 952.74445)
12,13-dihydroxy-11-methoxy-9-octadecenoic acid	7.05E-07	6.64E-06	C19 H36 O5	(343.24857, 29326.818)(344.25186, 5444.0503)(345.2543, 1107.0857)
C21 H43 N5 O14 S	4.97E-08	5.46E-07	C21 H43 N5 O14 S,	(620.2465, 30177.125)(621.2497, 6705.9004)(622.2501, 1974.6733)(623.2506, 857.6)
C7 H4 N4 O2	3.08E-15	2.03E-13	C7 H4 N4 O2	(175.0264, 18534.426)(176.0298, 1262.8212)(177.04199, 986.2334)
4-Hydroxyphenylacetylglutamine	3.35E-11	5.53E-10	C13 H15 N O6	(561.1707, 1312.5)(562.1741, 728.3)(280.08252, 16510.367)(281.08566, 2181.4)(282.087, 647.2)
Flucarbazone	2.21E-12	4.86E-11	C12 H11 F3 N4 O6 S	(395.0288, 21055.309)(396.03192, 2984.071)(397.03, 1240.1857)
C13 H16 O9	4.94E-11	6.53E-10	C13 H16 O9	(631.1498, 1001.6)(315.07178, 13295.311)(316.0753, 1900.3667)(317.0764, 697.2)

Table 2.4 Fold Change analysis results of 'tissue vs. discoloration' interpreting BH against control in negative mode (F = flesh, H = heart, BH = discoloured samples, control = C no discoloured samples) ('Experiment A').

Compound	Replication				Relative abundance normalized			
	FLESH		HEART		FLESH		HEART	
	BH (3)	control (3)	BH (3)	control (3)	BH (3)	control (3)	BH (3)	control (3)
2-Methylcitric acid	0	3	0	3	0	15.991	0	15.555
Glutathione, oxidized	1	3	0	3	5.195	16.763	0	16.301
C8 H7 N3 O S	3	3	3	0	19.826	20.153	19.852	0
alpha-D-Galactosyl-(1,1')-sn-glycerol 3-phosphate	0	2	0	3	0	11.669	0	16.690
Guanosine 3'-phosphate	1	3	0	3	5.408	16.939	0	17.099
Methylisocitric acid	3	3	3	3	17.158	17.760	16.657	17.990
Pseudouridine 5'-phosphate	1	3	0	3	5.636	17.721	0	18.462
Chlorogenic Acid	0	1	0	3	0	4.860	0	15.514
5-Acetamidovalerate	0	1	0	3	0	5.067	0	15.873
C18 H24 O13	0	1	0	3	0	4.973	0	16.651
260.8468@3.7007272	0	3	0	1	0	16.278	0	5.602
Trp Asp Ile	2	3	0	3	10.227	15.892	0	15.634
5-O-Feruloylquinic acid	1	2	0	3	4.995	10.122	0	15.806
Glu Ala Trp	1	3	0	3	4.944	15.461	0	15.584
C29 H42 N10 O9	0	2	1	3	0	10.388	5.098	16.203
9S,10S,11R-trihydroxy-12Z-octadecenoic acid	0	3	1	3	0	17.376	5.913	17.822
12,13-dihydroxy-11-methoxy-9-octadecenoic acid	0	3	3	3	0	16.674	16.246	17.501
17-hydroxy-linolenic acid	0	2	1	3	0	11.226	5.342	17.103
9-HOTE	0	2	1	3	0	11.432	5.289	16.970
C21 H43 N5 O14 S	3	0	3	3	15.218	0	17.197	16.036
C7 H4 N4 O2	0	0	0	3	0	0	0	15.248
4-Hydroxyphenylacetylglutamine	0	0	0	3	0	0	0	15.928
Flucarbazone	0	0	0	3	0	0	0	16.554

C13 H16 O9	0	0	0	3	0	0	0	15.544

Table 2.5 Analysis of Variance (ANOVA) results of 'tissue vs. discoloration' interpreting BC against control in negative mode ('Experiment A') ($P < 0.05$).

Compound	*p*	*p* (Corr)	CompositeSpectrum
C8 H10 O7	6.74E-13	3.77E-11	(217.03487, 12812.736)(218.03827, 1271.975)
2,5-Dioxopentanoate	0.00101	0.01414	(129.0191, 17871.6)(130.02242, 1193.9625)
Methylisocitric acid	0.003572	0.040011	(205.03491, 48789.254)(206.03839, 3224.3274)(207.04034, 498.02795)
Pseudouridine 5'-phosphate	9.49E-04	0.01414	(647.06256, 1771.7222)(648.0651, 926.0)(323.028, 40346.03)(324.03125, 3941.0098)(325.03256, 842.5667)
C7 H4 N4 O2	3.76E-12	1.05E-10	(175.0264, 18534.426)(176.0298, 1262.8212)(177.04199, 986.2334)

Table 2.6 Fold Change analysis results of 'tissue vs. discoloration' interpreting BC against control in negative mode (F = flesh, H = heart, BC = discoloured samples, control = C no discoloured samples) ('Experiment A').

Compound	Replication				Relative abundance normalized			
	FLESH		HEART		FLESH		HEART	
	BC (3)	control (3)	BC (3)	control (3)	BC (3)	control (3)	BC (3)	control (3)
C8 H10 O7	0	3	0	0	0	16.204	0	0
2,5-Dioxopentanoate	0	2	0	3	0	10.518	0	16.002
Pseudouridine 5'-phosphate	1	3	0	3	5.943	17.721	0	18.462
C7 H4 N4 O2	3	0	3	3	15.752	0	16.043	15.248

Table 2.7 Analysis of Variance (ANOVA) results of 'tissue vs. discoloration' interpreting BCL against control in negative mode ('Experiment A') ($P < 0.05$).

Compound	*p*	*p* (Corr)	Formula	Composite Spectrum
C21 H28 N8 O17	7.43E-15	2.04E-13		(663.15076, 24864.809)(664.15326, 5378.492)(665.1545, 1303.7916)
2-Amino-5-formylamino-6-(5-phospho-D-ribosylamino)pyrimidin-4(3H)-one	2.11E-13	2.69E-12	C10 H16 N5 O9 P	(761.12756, 5095.12)(762.1299, 1151.38)(380.06042, 15469.983)(381.06302, 1905.9751)
alpha-D-Galactosyl-(1,1')-sn-glycerol 3-phosphate	0.001	0.005	C9 H19 O11 P	(667.12366, 1148.2999)(333.0588, 20197.676)(334.06216, 2089.65)(335.063, 663.4)
2,5-Dioxopentanoate	0.001	0.005	C5 H6 O4	(129.0191, 17871.6)(130.02242, 1193.9625)
Guanosine 3'-phosphate	1.93E-14	3.54E-13	C10 H14 N5 O8 P	(725.1063, 1270.1124)(726.1094, 559.1)(362.0502, 22461.398)(363.05295, 2656.1365)(364.05484, 663.07996)
Pseudouridine 5'-phosphate	7.20E-15	2.04E-13	C9 H13 N2 O9 P	(647.06256, 1771.7222)(648.0651, 926.0)(323.028, 40346.03)(324.03125, 3941.0098)(325.03256, 842.5667)
2',3'-Cyclic UMP	2.53E-13	2.69E-12	C9 H11 N2 O8 P	(611.0418, 2377.9998)(612.04486, 832.10004)(305.0175, 37847.59)(306.02066, 3428.1833)(307.02246, 691.5751)
Molybdopterin precursor Z	2.93E-13	2.69E-12	C10 H12 N5 O7 P	(689.08514, 2331.6816)(690.0879, 829.45996)(344.03958, 37518.56)(345.04245, 4088.4414)(346.04456, 716.07)
404.168@4.9276795	0.003	0.012		(807.32623, 578.0)(403.1607, 14756.6875)(404.16388, 2822.1719)(405.16583, 713.1375)
9S,10S,11R-trihydroxy-12Z-octadecenoic acid	2.14E-09	1.47E-08	C18 H34 O5	(659.4713, 1245.95)(329.23288, 43508.137)(330.23633, 4656.7456)(331.2387, 863.55005)
12,13-dihydroxy-11-methoxy-9-octadecenoic acid	6.72E-10	5.28E-09	C19 H36 O5	(343.24857, 29326.818)(344.25186, 5444.0503)(345.2543, 1107.0857)
9-HOTE	0.001	0.005	C18 H30 O3	(293.21185, 29187.7)(294.2152, 4855.69)(295.2183, 791.8)
9-HOTE - 12.073699	0.001	0.005	C18 H30 O3	(293.21185, 25665.92)(294.21527, 4672.58)

Table 2.8 Fold Change analysis results of 'tissue vs. discoloration' interpreting BCL against control in negative mode (F = flesh, H = heart, pith = discoloured samples, control = C no discoloured samples) ('Experiment A').

Compound	Replication				Relative abundance normalized			
	FLESH		HEART		FLESH		HEART	
	BCL (3)	control (3)	BCL (3)	control (3)	BCL (3)	control (3)	BCL (3)	control (3)
C21 H28 N8 O17	0	3	0	3	0	15.863	0	15.681
2-Amino-5-formylamino-6-(5-phospho-D-ribosylamino)pyrimidin-4(3H)-one	0	3	0	3	0	15.850	0	15.862
alpha-D-Galactosyl-(1,1')-sn-glycerol 3-phosphate	0	2	0	3	0	11.669	0	16.690
2,5-Dioxopentanoate	0	2	0	3	0	10.518	0	16.002
Guanosine 3'-phosphate	0	3	0	3	0	16.939	0	17.099
Pseudouridine 5'-phosphate	0	3	0	3	0	17.721	0	18.462
2',3'-Cyclic UMP	0	3	0	3	0	17.800	0	17.817
Molybdopterin precursor Z	0	3	0	3	0	17.309	0	17.243
9S,10S,11R-trihydroxy-12Z-octadecenoic acid	0	3	0	3	0	17.376	0	17.822
12,13-dihydroxy-11-methoxy-9-octadecenoic acid	0	3	0	3	0	16.674	0	17.501
9-HOTE	0	2	0	3	0	11.226	0	17.103
9-HOTE - 12.073699	0	2	0	3	0	11.432	0	16.970

Table 2.9 Analysis of Variance (ANOVA) results of 'tissue vs. discoloration' interpreting pith against control in negative mode ('Experiment A') ($P < 0.05$).

Compound	*p*	*p* (Corr)	Formula	Composite Spectrum
387.9458@0.6116207	0.005	0.041		(386.93845, 14434.013)(387.9397, 6372.4614) (388.93723, 6966.303) (389.93802, 2086.1667) (390.93564, 1153.0834)
488.1899@0.94757694	3.04E-04	0.006		(487.18265, 72510.4)(488.17792, 30378.389)(489.18094, 4938.0034) (490.1774, 626.7471)
196.0601@0.9854668	0.005	0.041		(195.05287, 82119.03) (196.0563, 4263.237) (197.05734, 440.69666)
C8 H7 N5 O2 S	0.003	0.031	C8 H7 N5 O2 S	(236.0257, 16011.329) (237.02875, 1384.5077) (238.023, 798.1167)
5-(3-Pyridyl)-2-hydroxytetrahydrofuran	0.003	0.031	C9 H11 N O2	(164.07138, 30651.727) (165.07472, 2771.31)
2,5-Dioxopentanoate	0.001	0.016	C5 H6 O4	(129.0191, 17871.6) (130.02242, 1193.9625)
206.0425@2.4050667	0.007	0.049		(205.03491, 48789.254) (206.03839, 3224.3274) (207.04034, 498.02795)
Chlorogenic Acid	0.003	0.031		(386.93845, 14434.013) (387.9397, 6372.4614) (388.93723, 6966.303)(389.93802, 2086.1667) (390.93564, 1153.0834)
176.0336@1.026842	1.38E-11	9.00E-10		(487.18265, 72510.4) (488.17792, 30378.389)(489.18094, 4938.0034) (490.1774, 626.7471)
4-Hydroxyphenylacetylglutamine	3.35E-11	1.09E-09		(195.05287, 82119.03) (196.0563, 4263.237) (197.05734, 440.69666)

Table 2.10 Fold Change analysis results of 'tissue vs. discoloration' interpreting pith against control in negative mode (F = flesh, H = heart, pith = discoloured samples, control = C no discoloured samples) ('Experiment A').

Compound	Replication				Relative abundance			
	FLESH		HEART		FLESH		HEART	
	PITH (3)	Control (3)	PITH (3)	Control (3)	PITH (3)	Control (3)	PITH (3)	Control (3)
196.0601@0.9854668	3	3	3	3	18.772	18.437	18.757	17.732
C8 H7 N5 O2 S	0	1	0	3	0	5.051	0	15.744
5-(3-Pyridyl)-2-hydroxytetrahydrofuran	3	3	3	3	15.820	16.465	16.037	17.279
2,5-Dioxopentanoate	0	2	0	3	0	10.518	0	16.002
Chlorogenic Acid	0	1	0	3	0	4.859	0	15.514
176.0336@1.026842	3	0	3	3	15.291	0	15.510	15.248
4-Hydroxyphenylacetylglutamine	0	0	0	3	0	0	0	15.928

3. Metabolomic differences between affected and non-affected potato tuber tissues (positive mode).

Table 3.1 Analysis of Variance (ANOVA) results of 'discoloration only' interpretation in positive mode ('Experiment A') ($P < 0.001$).

Compound	*p*	*p* (Corr)	Formula	Composite Spectrum
C7 H13 N	5E-05	2.52E-04	C7 H13 N	(112.11221, 16524.17)(113.11553, 1710.6615)
Monoethylglycylxylidide (MEGX)	7E-08	1.03E-06	C12 H18 N2 O	(207.14925, 49947.812)(208.15257, 6164.3467)(209.15547, 286.1375)
1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine N-oxide	1E-08	3.16E-07	C12 H15 N O	(190.12265, 15006.92)(191.12604, 2116.93)
C8 H9 N	4E-06	3.78E-05		(120.08117, 53939.76)(121.08425, 5255.4)
Val Ile	2E-05	1.17E-04	C11 H22 N2 O3	(231.1705, 36542.703)(232.17377, 4246.4185)(233.17535, 321.44998)
8-Hydroxyadenine	8E-05	3.58E-04	C5 H5 N5 O	(152.05661, 23138.9)(153.05951, 1572.62)
4-Nitrotoluene	1E-06	1.39E-05	C7 H7 N O2	(138.05505, 22050.74)(139.05832, 1842.0137)(140.07065, 1480.355)
N-(6-aminohexanoyl)-6-aminohexanoic acid	2E-05	1.54E-04	C12 H24 N2 O3	(489.3659, 568.0)(245.18631, 50651.594)(246.18947, 6554.863)(247.19153, 577.7714)
N-(6-aminohexanoyl)-6-aminohexanoic acid + 3.180625	3E-05	1.59E-04	C12 H24 N2 O3	(245.18628, 46456.223)(246.1895, 5928.938)(247.1918, 333.35715)
C9 H6 O3	2E-07	2.62E-06		(163.04074, 207204.16)(164.04231, 22030.295)(165.04445, 231.05)
Chlorogenic Acid	2E-07	2.98E-06	C16 H18 O9	(355.10284, 162831.03)(356.10632, 26187.186)(357.1086, 3695.96)(358.1106, 0.0)
6Z-Octene-2,4-diynoic acid	2E-05	1.32E-04	C8 H6 O2	(135.04405, 18477.742)(136.04747, 1723.6385)
Trp Asp Gly	1E-04	4.46E-04	C17 H20 N4 O6	(377.1459, 35824.555)(378.14902, 6506.681)(379.15155, 1193.5636)
4'-Prenyloxyresveratrol	5E-10	1.99E-08	C19 H20 O4	(335.1244, 26671.963)(336.12753, 4794.383)(337.13058, 930.64703)
Gln Phe Gln	9E-05	3.89E-04	C19 H27 N5 O6	(422.20282, 15792.631)(423.2058, 3327.8804)(424.208, 793.5133)
817.042@5.688111	7E-06	6.33E-05		(818.0494, 24823.934)(819.05054, 1309.6777)
1434.9309@6.42445	2E-05	1.17E-04		(1435.9357, 18127.955)(1436.936, 5114.44)(1437.9336, 748.3769)
2151.3877@6.424066	9E-05	3.92E-04		(1076.702, 23193.475)(1077.2034, 29283.441)(1077.7037, 14523.835)(1078.2039, 4369.65)(1078.7034, 1132.5311)
9S,10S,11R-trihydroxy-12Z-octadecenoic acid	2E-04	7.93E-04	C18 H34 O5	(705.4522, 2004.3251)(706.4565, 957.1667)(353.23062, 66095.28)(354.234, 12574.334)(355.2363, 1722.6445)(356.2396,

				274.26666)
(2-Chlorophenyl)diphenylmethane	7E-05	3.06E-04	C19 H15 Cl	(557.1802, 957.9704)(279.09384, 48525.977)(280.09717, 8713.833)(281.10025, 839.91003)
Spisulosine	3E-05	1.59E-04	C18 H39 N O	(571.61475, 1817.9598)(572.6175, 820.93036)(286.31107, 78243.87)(287.3145, 14986.226)(288.3175, 806.38684)
16,16-dimethyl-PGD2	1E-09	3.43E-08	C22 H36 O5	(381.2627, 20472.072)(382.2661, 4348.2285)(383.26865, 845.9715)
2-amino-14,16-dimethyloctadecan-3-ol	1E-06	1.39E-05	C20 H43 N O	(627.6791, 576.2)(314.34247, 56993.42)(315.3459, 11512.849)(316.3487, 840.04333)
Lagochilin	2E-04	7.29E-04	C20 H36 O5	(379.2466, 24277.55)(380.24994, 4979.38)
8-methoxy-13-hydroxy-9,11-octadecadienoic acid	9E-06	7.19E-05	C19 H34 O4	(349.23602, 24144.957)(350.2393, 4728.2773)(351.2422, 944.98)
12-amino-octadecanoic acid	3E-05	1.59E-04	C18 H37 N O2	(300.2904, 24718.33)(301.29358, 4857.763)(302.2989, 777.73083)
C25 H49 N5 O2	3E-04	9.94E-04	C25 H49 N5 O2	(452.3956, 57907.793)(453.39902, 13081.449)(454.40134, 2290.8867)(455.40295, 0.0)
Dodemorph	6E-05	2.93E-04	C18 H35 N O	(563.55206, 1530.7234)(564.55505, 715.265)(282.2797, 140367.92)(283.28308, 24132.012)(284.28714, 2525.237)
C30 H59 N5 O4	1E-04	5.40E-04	C30 H59 N5 O4	(554.4642, 68221.51)(555.46735, 19890.814)(556.469, 4844.114)(557.4708, 27.959997)
6Z-Octene-2,4-diynoic acid + 1.3391001	1E-04	5.12E-04	C8 H6 O2	(135.04404, 22536.86)(136.04709, 2306.5803)(137.05966, 2287.55)
PRIMA-1	1E-06	1.39E-05	C9 H15 N O3	(186.11247, 14745.975)(187.11568, 1640.4875)
Trp Ser Gln	2E-05	1.17E-04	C19 H25 N5 O6	(420.1867, 20032.28)(421.18988, 3922.0715)(422.19165, 982.5999)
Mometasone Furoate	3E-05	1.59E-04	C27 H30 Cl2 O6	(543.1333, 24000.436)(544.13635, 4786.8335)(545.1345, 2484.1445)(546.1367, 771.0)
N-Hydroxypentobarbital	2E-05	1.36E-04	C11 H18 N2 O4	(243.1341, 32700.79)(244.1372, 4582.067)(245.13857, 849.5)
Val Ile + 1.5134287	2E-10	7.90E-09	C11 H22 N2 O3	(231.17049, 43499.727)(232.17354, 4824.7144)(233.17577, 468.43332)
Val Ile + 1.953625	3E-08	4.87E-07	C11 H22 N2 O3	(231.1705, 38453.688)(232.17377, 4701.0625)(233.17572, 411.6333)
Adenine	3E-34	4.53E-32	C5 H5 N5	(136.06184, 189277.8)(137.06474, 12738.7)
N-(6-aminohexanoyl)-6-aminohexanoic acid + 2.6753333	7E-29	4.98E-27	C12 H24 N2 O3,	(245.18616, 48491.12)(246.18939, 6382.033)(247.19185, 237.95)
Succinoadenosine	1E-08	3.04E-07	C14 H17 N5 O8	(384.11508, 47009.523)(385.11804, 7227.0747)(386.11987, 1015.0667)
1508.1241@4.6621003	5E-05	2.52E-04		(755.0697, 19357.41)(755.5708, 14757.07)(756.07166,

				2031.3102)(1509.132, 795.4)
816.8409@5.68775	6E-08	9.68E-07		(817.84875, 22885.361)(818.8509, 3258.9375)
9-HOTE	6E-05	2.93E-04	C18 H30 O3	(295.2272, 42787.54)(296.23044, 8152.69)(297.2333, 1323.525)

Table 3.2 Fold Change analysis results of 'discoloration only' interpretation in positive mode ('Experiment B')

Compound	Replication					Relative abundance normalized				
	BC (6)	BCL (6)	BH (6)	control (6)	PITH (6)	BC (6)	BCL (6)	BH (6)	control (6)	PITH (6)
C7 H13 N	6	6	2	6	6	16.562	16.748	5.533	16.704	16.766
1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine N-oxide	0	4	0	6	0	0	10.315	0	15.822	0
C8 H9 N	6	0	5	2	6	17.712	0	14.326	5.856	17.523
Val Ile	2	0	6	6	2	5.491	0	16.978	16.618	5.398
8-Hydroxyadenine	2	0	0	6	2	5.563	0	0.000	16.542	5.376
4-Nitrotoluene	5	5	0	6	6	13.297	13.502	0.000	16.385	16.072
N-(6-aminohexanoyl)-6-aminohexanoic acid	2	0	6	6	2	5.710	0.000	17.087	17.135	5.674
N-(6-aminohexanoyl)-6-aminohexanoic acid + 3.180625	2	0	6	6	2	5.637	0	16.406	17.149	5.663
C9 H6 O3	6	6	6	6	6	19.536	19.803	18.384	19.621	19.856
Chlorogenic Acid	6	6	6	6	6	19.108	19.401	17.925	19.273	19.526
6Z-Octene-2,4-diynoic acid	6	6	2	6	6	15.907	16.147	5.095	15.971	16.236
Trp Asp Gly	1	1	6	3	0	2.601	2.634	17.239	7.760	0.000
4'-Prenyloxyresveratrol	5	6	0	6	6	13.123	16.101	0.000	16.449	16.284
Gln Phe Gln	4	6	0	6	4	10.183	15.346	0.000	15.563	10.419
817.042@5.688111	2	0	6	1	0	5.318	0.000	16.430	2.556	0.000
1434.9309@6.42445	5	6	0	3	6	13.132	15.916	0.000	7.890	15.986
9S,10S,11R-trihydroxy-12Z-octadecenoic acid	6	6	6	6	6	16.851	16.742	18.947	17.107	17.025
16,16-dimethyl-PGD2	0	0	1	6	0	0	0	2.687	15.999	0.000
Lagochilin	1	0	1	6	2	2.647	0	2.628	16.701	5.378
8-methoxy-13-hydroxy-9,11-octadecadienoic acid	1	0	0	6	2	2.699	0	0	16.955	5.480
6Z-Octene-2,4-diynoic acid + 1.3391001	2	6	0	0	2	5.459	15.991	0	0	5.455
PRIMA-1	0	6	0	1	1	0	15.649	0	2.579	2.602
Trp Ser Gln	4	6	0	0	4	10.321	16.136	0	0	10.443
Mometasone Furoate	1	1	6	0	1	2.715	2.796	17.767	0	2.781

N-Hydroxypentobarbital	1	0	6	1	1	2.586	0	16.776	2.559	2.546
Val Ile + 1.5134287	0	0	6	1	0	0	0	17.289	2.628	0
Val Ile + 1.953625	0	0	6	2	0	0	0	17.126	5.284	0
Adenine	0	0	6	0	0	0	0	19.281	0	0
N-(6-aminohexanoyl)-6-aminohexanoic acid + 2.6753333	0	0	6	0	0	0	0	16.720	0	0
Succinoadenosine	0	2	6	0	0	0	5.065	17.103	0	0
1508.1241@4.6621003	2	0	6	2	0	5.388	0	16.871	5.404	0
816.8409@5.68775	2	0	6	0	0	5.280	0	16.305	0	0
9-HOTE	1	2	6	0	1	2.540	5.124	17.310	0	2.547

Table 3.3 Analysis of Variance (ANOVA) results of 'tissue vs. discoloration' interpreting BH against control in positive mode ('Experiment B') ($P < 0.001$).

Compound	*p*	*p* (Corr)	Formula	Composite Spectrum
2073.8328@4.793522	8E-11	1.74E-09		(1037.9235, 20394.744)(1038.4241, 13987.85)(1038.9247, 4981.226)(1039.424, 1376.9783)
C18 Sulfatide	6E-11	1.36E-09	C42 H81 N O11 S,	(830.54034, 20169.791)(831.54114, 2783.137)
12,13-dihydroxy-11-methoxy-9-octadecenoic acid	9E-08	1.58E-06	C19 H36 O5	(733.48474, 750.45)(367.24646, 37451.957)(368.2496, 6978.0605)(369.24722, 1903.4624)
12,13-dihydroxy-11-methoxy-9-octadecenoic acid + 9.8632	7E-09	1.40E-07	C19 H36 O5	(367.24643, 34464.94)(368.24988, 6678.98)(369.25198, 1188.2571)
(S)-lamenallenic acid	5E-06	8.89E-05	C18 H30 O2	(279.23303, 76858.305)(280.2359, 13619.056)(281.23938, 1555.1112)
C29 H23 N21 O3	1E-14	8.82E-13		(714.23596, 14728.709)(715.2389, 5891.07)(716.2413, 1560.1799)
Glu Met	8E-17	1.78E-14	C10 H18 N2 O5 S	(279.1012, 15337.546)(280.10422, 1992.4543)(281.0991, 848.1272)
5-Methoxydimethyltryptamine	8E-12	2.10E-10	C13 H18 N2 O	(219.14948, 20214.021)(220.1526, 2757.1667)
C11 H17 N O8 S	2E-19	1.07E-16		(324.07523, 14398.645)(325.07837, 1920.9364)(326.07388, 892.5181)
Valinopine	1E-13	5.91E-12	C10 H17 N O6,	(248.1132, 15463.818)(249.11649, 1942.8)
4-Hydroxyphenylacetylglutamine	3E-11	6.82E-10	C13 H15 N O6	(282.0974, 17683.637)(283.10062, 2464.25)(284.1034, 740.4)
Alpha-CEHC	2E-08	3.88E-07	C16 H22 O4	(279.15973, 96221.29)(280.1651, 18032.219)(281.16803, 2110.4268)
Ile Ala	1E-13	5.91E-12	C9 H18 N2 O3	(203.13914, 18824.967)(204.14233, 1881.1334)
6-Acetamido-3-aminohexanoate	4E-16	5.82E-14	C8 H16 N2 O3	(189.12352, 15179.101)(190.12682, 1473.0)
Val Ile + 1.8330001	3E-12	7.89E-11	C11 H22 N2 O3	(231.17029, 31731.766)(232.1736, 3670.2666)(233.1759, 675.0)
Leu Arg Pro	1E-12	4.36E-11	C17 H32 N6 O4	(407.23904, 17722.533)(408.24228, 3631.7332)(409.2435, 996.53326)
N-Hydroxypentobarbital + 4.3743334	4E-10	8.94E-09	C11 H18 N2 O4	(485.26065, 2703.1667)(486.26364, 1046.1)(243.13383, 28760.0)(244.1368, 3315.3333)(245.1491, 1537.2)
4-oxo-nonenal	4E-11	9.83E-10	C9 H14 O2	(155.10664, 18979.666)(156.10986, 2294.0)

528.5197@2.4804444	3E-13	1.05E-11		(265.26718, 19381.367)(265.76636, 683.1889)(266.269, 788.55707)
C8 H4 O	3E-15	2.48E-13		(117.03357, 14132.089)(118.0376, 1367.4443)
C10 H11 N5 O2	8E-12	2.13E-10		(234.09811, 16687.02)(235.10132, 1828.1199)
IU1	1E-13	5.91E-12	C18 H21 F N2 O	(301.1713, 34285.8)(302.1746, 5803.467)(303.17673, 885.5667)
C21 H33 N O13	3E-13	1.03E-11		(508.2028, 18153.941)(509.206, 4278.3203)(510.2082, 1189.96)
C14 H23 N	1E-13	5.91E-12		(206.19048, 15508.532)(207.19392, 2677.3665)
n-Pentadecylamine	2E-14	1.46E-12	C15 H33 N	(228.26886, 18312.525)(229.27234, 2900.6)
1-Octadecanamine	8E-16	8.44E-14	C18 H39 N,	(270.3159, 14056.574)(271.31915, 2925.0)

Table 3.4 Fold Change analysis results of 'tissue vs. discoloration' interpreting BH against control in positive mode (F = flesh, H = heart, BH = discoloured samples, control = no discoloured samples) ('Experiment B').

Comound	Replication				Relative abundance normalized			
	FLESH		HEART		FLESH		HEART	
	BH (3)	Control (3)	BH (3)	Control (3)	BH (3)	Control (3)	BH (3)	Control (3)
2073.8328@4.793522	0	3	3	3	0	16.470	16.146	17.196
C18 Sulfatide	0	3	3	3	0	15.796	15.497	16.470
12,13-dihydroxy-11-methoxy-9-octadecenoic acid	0	3	3	3	0	17.510	16.538	18.175
12,13-dihydroxy-11-methoxy-9-octadecenoic acid + 9.8632	0	3	3	3	0	17.621	16.563	17.459
(S)-lamenallenic acid	0	3	3	3	0	18.100	17.866	19.134
C29 H23 N21 O3	0	0	0	3	0	0	0	16.022
Glu Met	0	0	0	3	0	0	0	15.659
5-Methoxydimethyltryptamine	0	0	0	3	0	0	0	15.650
C11 H17 N O8 S	0	0	0	3	0	0	0	15.537
Valinopine	0	0	0	3	0	0	0	15.366
4-Hydroxyphenylacetylglutamine	0	0	0	3	0	0	0	15.937
Alpha-CEHC	3	0	3	3	18.692	0	18.895	15.721
Ile Ala	0	0	3	0	0	0	15.625	0
6-Acetamido-3-aminohexanoate	0	0	3	0	0	0	15.443	0
Val Ile + 1.8330001	0	0	3	0	0	0	16.755	0
Leu Arg Pro	0	0	3	0	0	0	16.544	0
N-Hydroxypentobarbital + 4.3743334	0	0	3	0	0	0	16.379	0
4-oxo-nonenal	0	0	3	0	0	0	16.221	0.000
528.5197@2.4804444	0	0	0	3	0	0	0	15.834
C8 H4 O	0	0	0	3	0	0	0	15.672
C10 H11 N5 O2	0	0	0	3	0	0	0	15.621
IU1	0	0	0	3	0	0	0	16.407
C21 H33 N O13	3	0	0	0	16.793	0	0	0

C14 H23 N	3	0	0	0	15.935	0	0	0
n-Pentadecylamine	3	0	0	0	15.977	0	0	0
1-Octadecanamine	3	0	0	0	15.842	0	0	0

Table 3.5 Analysis of Variance (ANOVA) results of 'tissue vs. discoloration' interpreting BC against control in positive mode ('Experiment B') ($P < 0.001$).

Compound	*p*	*p* (Corr)	Formula	Composite Spectrum
Lomustine	2E-12	2.26E-10	C9 H16 Cl N3 O2	(256.08197, 19921.65)(257.0854, 2570.0999)(258.0873, 944.7)
Valinopine	1E-13	2.37E-11	C10 H17 N O6	(248.1132, 15463.818)(249.11649, 1942.8)
C14 H5 N O17	5E-12	4.62E-10		(459.96353, 15804.37)(460.96613, 847.91425)
Alpha-CEHC	3E-08	1.51E-06	C16 H22 O4	(279.15973, 96221.29)(280.1651, 18032.219)(281.16803, 2110.4268)
C10 H11 N5 O2	8E-12	5.79E-10		(234.09811, 16687.02)(235.10132, 1828.1199)
IU1	1E-13	2.37E-11	C18 H21 F N2 O,	(301.1713, 34285.8)(302.1746, 5803.467)(303.17673, 885.5667)
C22 H46 N16 O10	1E-07	6.26E-06		(348.18674, 11900.898)(348.68808, 4870.863)(349.18958, 2343.25)(695.36554, 89979.6)(696.3688, 23032.602)(697.3715, 4799.22)(698.3741, 529.0)

Table 3.6 Fold Change analysis results of 'tissue vs. discoloration' interpreting BC against control in positive mode (F = flesh, H = heart, BC = discoloured samples, control = no discoloured samples) ('Experiment B').

Compound	Replication				Relative abundance normalized			
	FLESH		HEART		FLESH		HEART	
	BC (3)	Control (3)	BC (3)	Control (3)	BC (3)	Control (3)	BC (3)	Control (3)
Lomustine	0	3	0	0	0	17.049	0	0
Valinopine	0	0	0	3	0	0	0	15.366
C14 H5 N O17	0	0	0	3	0	0	0	14.878
Alpha-CEHC	3	0	3	3	18.296	0	18.584	15.721
C10 H11 N5 O2	0	0	0	3	0	0	0	15.621
IU1	0	0	0	3	0	0	0	16.407
C22 H46 N16 O10	0	0	3	0	0	0	18.284	0

Table 3.7 Analysis of Variance (ANOVA) results of 'tissue vs. discoloration' interpreting BCL against control in positive mode ('Experiment B') ($P < 0.01$).

Compound	*p*	*p* (Corr)	Formula	Composite Spectrum
C12 H26 N2 O14 S	5.18E-09	1.82E-07	C12 H26 N2 O14 S	(455.11707, 21752.926)(456.1205, 3396.4873)(457.1174, 1931.1)
Lomustine	1.93E-12	1.24E-10	C9 H16 Cl N3 O2	(256.08197, 19921.65)(257.0854, 2570.0999)(258.0873, 944.7)
958.6047@4.348375	6.8E-10	3.29E-08		(480.3097, 16261.899)(480.81125, 7980.7505)(481.31244, 2630.8499)(481.8134, 880.02)
Nitrothal-isopropyl	9.32E-10	4.01E-08	C14 H17 N O6	(318.09576, 34267.65)(319.09897, 4055.0786)(320.09503, 2954.4714)(321.09747, 209.9)
C11 H21 N O6	1.71E-12	1.24E-10	C11 H21 N O6	(264.14438, 34130.7)(265.14783, 4123.725)(266.15063, 1072.9333)
C12 H15 N5 O	1.3E-12	1.24E-10	C12 H15 N5 O	(246.13443, 30592.441)(247.13713, 4019.77)
C10 H13 N O6	2.02E-11	1.12E-09	C10 H13 N O6	(244.08171, 17919.473)(245.08467, 2371.543)
IU1	1.34E-13	2.15E-11	C18 H21 F N2 O	(301.1713, 34285.8)(302.1746, 5803.467)(303.17673, 885.5667)
C13 H20 N2 O3	1.15E-09	4.45E-08	C13 H20 N2 O3	(253.15501, 34583.86)(254.15826, 4913.45)(255.16084, 554.56665)
1001.7602@6.873572	1.12E-16	4.34E-14		(1002.767, 17980.014)(1003.7674, 1756.6858)(1004.76776, 1774.8)
Ganglioside GQ1c (d18:1/24:0)	1.66E-13	2.15E-11	C113 H195 N5 O55	(1252.14, 1599.557)(1252.6398, 978.82855)(1253.143, 23835.514)(1253.644, 10852.4)

Table 3.8 Fold Change analysis results of 'tissue vs. discoloration' interpreting BCL against control in positive mode (F = flesh, H = heart, BCL = discoloured samples, control = no discoloured samples) ('Experiment B').

Compound	Replication				Relative abundance normalized			
	FLESH		HEART		FLESH		HEART	
	BCL (3)	Control (3)	BCL (3)	Control (3)	BCL (3)	Control (3)	BCL (3)	Control (3)
C12 H26 N2 O14 S	3	0	0	0	0	16.82	0	0
Lomustine	3	0	0	0	0	17.05	0	0
958.6047@4.348375	0	3	0	0	16.34	0	0	0
Nitrothal-isopropyl	0	0	3	0	0	0	0	16.72
C11 H21 N O6	0	0	3	0	0	0	0	17.62
C12 H15 N5 O	0	0	3	0	0	0	0	17.126
C10 H13 N O6	0	0	3	0	0	0	0	16.86
IU1	0	0	3	0	0	0	0	16.46
C13 H20 N2 O3	0	0	0	3	0	0	15.96	0
1001.7602@6.873572	0	0	0	3	0	0	16.87	0
Ganglioside GQ1c (d18:1/24:0)	0	0	0	3	0	0	17.297	0

Table 3.9 Analysis of Variance (ANOVA) results of 'tissue vs. discoloration' interpreting pith against control in positive mode ('Experiment B') ($P < 0.01$).

Compound	*p*	*p* (Corr)	Formula	Composite Spectrum
851.3623@5.9376326	4E-13	1.72E-11		(852.36926, 19522.111)(853.3704, 7034.422)
851.7631@5.937048	4E-13	1.72E-11		(852.77, 23227.572)(853.77057, 1944.3)
851.9631@5.9375796	4E-13	1.73E-11		(852.9701, 18184.31)(853.9697, 974.38947)
2153.424@6.879866	7E-15	1.15E-12		(1077.7179, 14149.221)(1078.2183, 16672.307)(1078.7185, 8686.773)(1079.219, 2725.68)(1079.718, 900.1923)
C29 H23 N21 O3	1E-14	1.40E-12		(714.23596, 14728.709)(715.2389, 5891.07)(716.2413, 1560.1799)
Glu Met	8E-17	2.82E-14	C10 H18 N2 O5 S	(279.1012, 15337.546)(280.10422, 1992.4543)(281.0991, 848.1272)
Valinopine	1E-13	7.84E-12	C10 H17 N O6	(248.1132, 15463.818)(249.11649, 1942.8)
4-Hydroxyphenylacetylglutamine	3E-11	8.13E-10	C13 H15 N O6	(282.0974, 17683.637)(283.10062, 2464.25)(284.1034, 740.4)
C14 H5 N O17	5E-12	1.84E-10		(459.96353, 15804.37)(460.96613, 847.91425)
2003.518@6.871636	8E-14	6.87E-12		(1002.767, 17770.717)(1003.2674, 3370.2092)(1003.7688, 1985.5819)(1004.2592, 1084.5667)
IU1	1E-13	7.84E-12	C18 H21 F N2 O	(301.1713, 34285.8)(302.1746, 5803.467)(303.17673, 885.5667)
Gly His Val	2E-11	5.86E-10	C13 H21 N5 O4	(312.16754, 15306.708)(313.17056, 2331.93)

Table 3.10 Fold Change analysis results of 'tissue vs. discoloration' interpreting pith against control in positive mode (F = flesh, H = heart, pith = discoloured samples, control = no discoloured samples) ('Experiment B').

Compound	Replication				Relative abundance normalized			
	FLESH		HEART		FLESH		HEART	
	PITH (3)	Control (3)	PITH (3)	Control (3)	PITH (3)	Control (3)	PITH (3)	Control (3)
851.3623@5.9376326	3	3	3	0	16.707	16.222	15.913	0
851.7631@5.937048	3	3	3	0	16.607	16.160	15.854	0
851.9631@5.9375796	3	3	3	0	16.168	15.692	15.378	0
2153.424@6.879866	3	3	3	0	17.147	17.444	17.054	0
C29 H23 N21 O3	0	0	0	3	0	0	0	16.022
Glu Met	0	0	0	3	0	0	0	15.659
Valinopine	0	0	0	3	0	0	0	15.366
4-Hydroxyphenylacetylglutamine	0	0	0	3	0	0	0	15.937
C14 H5 N O17	0	0	0	3	0	0	0	14.878
2003.518@6.871636	3	0	0	0	16.743	0	0	0
IU1	0	0	0	3	0	0	0	16.407
Gly His Val	0	0	3	0	0	0	15.475	0

4. Metabolomic differences between control samples of potato stocks with different susceptibility to blackheart disorder (negative mode).

Table 4.1 Analysis of Variance (ANOVA) results of 'susceptibility only' interpretation in negative mode ('Experiment C') ($P < 0.05$).

Compound	*p*	*p* (Corr)	Formula	Composite Spectrum
C16 H8 N2 O S5	5E-04	0.008		(402.91592, 4538.849)(403.91714, 1594.766)(404.9145, 1628.9803)(405.91605, 584.85)
C11 H16 N6 O5	3E-03	0.033		(311.1112, 22844.135)(312.11325, 2631.6682)(313.1154, 744.75)
C4 H7 N O4	6E-03	0.045		(132.03162, 49180.18)(133.03499, 2025.3241)(134.036, 0.0)
C18 H21 N9 O7	4E-03	0.037		(474.14957, 222255.48)(475.15176, 35023.727)(476.15283, 7228.4507)(477.15527, 0.0)
C13 H20 O8 S	5E-03	0.042		(335.08066, 8133.3022)(336.08478, 1094.5541)(337.09085, 765.5231)
C7 H6 N4 O4	2E-03	0.026		(209.03165, 13910.506)(210.03493, 1056.8354)(211.0359, 655.1)
Hibiscetin	4E-03	0.037	C15 H10 O9	(333.02628, 9041.861)(334.0299, 1115.4205)(335.03427, 666.0667)
L-Glutamyl 5-phosphate	2E-04	0.005	C5 H10 N O7 P	(226.01141, 35382.715)(227.01447, 1955.8903)(228.0157, 602.94446)
Pantothenic Acid	1E-03	0.020	C9 H17 N O5	(437.21164, 1081.4546)(218.1025, 19389.06)(219.1056, 1966.6147)
5,7,3',4',5'-Pentahydroxy-3,6,8-trimethoxyflavone	3E-04	0.005	C18 H16 O10	(391.0683, 30746.295)(392.07144, 4133.4443)(393.07272, 1130.6053)
Quinic acid	8E-07	0.000	C7 H12 O6	(191.05536, 67761.15)(192.05875, 4338.9565)(193.05977, 334.8414)
C16 H24 N6 O5 S2	2E-03	0.026		(443.1178, 6778.3438)(444.12094, 1418.4585)(445.12354, 818.94995)
913.5031@5.753782	1E-06	0.000		(912.49445, 36849.215)(913.49725, 25095.98)(914.4994, 9113.316)(915.5012, 2277.2883)(916.5046, 0.0)
897.5062@5.808823	2E-05	0.001		(896.49884, 42223.406)(897.50214, 19562.84)(898.5044, 6187.704)(899.5067, 1663.0891)(900.5081, 0.0)

Table 4.2 Fold Change analysis results of 'susceptibility only' interpretation in negative mode (Y = susceptible stock 23, N = non-susceptible stock 12) ('Experiment C').

Compound	Replication		Relative abundance normalized	
	Y (18)	N (23)	Y (18)	N (23)
C13 H20 O8 S	14	23	10.856	14.482
C7 H6 N4 O4	18	16	15.950	10.524
Hibiscetin	18	16	15.206	10.302
5,7,3',4',5'-Pentahydroxy-3,6,8-trimethoxyflavone	18	23	18.177	17.137
Quinic acid	18	23	16.809	18.477
C16 H24 N6 O5 S2	18	16	14.335	9.445
913.5031@5.753782	9	23	7.204	16.376
897.5062@5.808823	11	23	8.786	16.566

Table 4.3 Analysis of Variance (ANOVA) results of 'tissue vs. susceptibility' interpretation in negative mode ('Experiment C') ($P < 0.01$).

Compound	*p*	*p* (Corr)	Formula	Composite Spectrum
387.9461@0.6401463	0.001	0.002		(386.93845, 11311.679)(387.93958, 5095.349)(388.937, 5651.838)(389.9377, 1845.4519)(390.93524, 1095.9216)
Ribose-1-arsenate	0.003	0.009	C5 H11 As O8	(272.95837, 50745.832)(273.95947, 14040.337)(274.95667, 14253.221)(275.95813, 2367.4463)(276.9552, 926.54755)
Benzal chloride	4.00E-07	4.14E-06	C7 H6 Cl2	(158.9781, 30128.83)(159.9794, 4503.735)(160.97606, 4210.422)
C17 H3 Cl N4 O S4	7.07E-05	2.72E-04	C17 H3 Cl N4 O S4,	(440.88077, 4518.3535)(441.882, 1532.4929)(442.87967, 2227.9023)(443.88184, 601.0643)
C4 H8 N2 O2 S4	3.53E-05	1.56E-04	C4 H8 N2 O2 S4	(242.93924, 6560.7393)(243.94067, 1230.7755)(244.9375, 1578.7319)
C4 H7 N O4	2.45E-05	1.27E-04	C4 H7 N O4	(132.03162, 49180.18)(133.03499, 2025.3241)(134.036, 0.0)
C18 H21 N9 O7	7.89E-05	2.72E-04	C18 H21 N9 O7	(474.14957, 222255.48)(475.15176, 35023.727)(476.15283, 7228.4507)(477.15527, 0.0)
Pantothenic Acid	7.71E-11	2.39E-09	C9 H17 N O5,	(437.21164, 1081.4546)(218.1025, 19389.06)(219.1056, 1966.6147)
C24 H6 N4 O9	4.76E-04	0.001477	C24 H6 N4 O9,	(493.00778, 3641.7913)(494.01028, 851.10913)
C17 H7 N7 O8	4.12E-06	3.20E-05	C17 H7 N7 O8,	(436.02872, 4793.353)(437.03183, 986.8)
969.4385@5.8075547	1.95E-05	1.21E-04		(968.42896, 5494.5835)(969.43097, 3245.0442)(970.43274, 1322.0187)(971.4337, 710.275)
887.4782@5.8079376	3.16E-08	4.90E-07		(886.46954, 3878.119)(887.4721, 1888.5938)(888.4685, 1700.3499)(889.4703, 876.3857)

Table 4.4 Fold Change analysis results of 'tissue vs. susceptibility' interpretation in negative mode (F = flesh, H = heart, Y = susceptible stock 23, N = non-susceptible stock 12) ('Experiment C').

Compound	Replication				Relative abundance normalized			
	FLESH		HEART		FLESH		HEART	
	N (12)	Y (8)	N (11)	Y (10)	N (12)	Y (8)	N (11)	Y (10)
887.4782@5.8079376	12	1	3	0	14.254	1.609	3.412	0

5. Metabolomic differences between control samples of potato stocks with different susceptibility to blackheart disorder (positive mode).

Table 5.1 Analysis of Variance (ANOVA) results of 'susceptibility only' interpretation in positive mode ('Experiment D') ($P < 0.05$).

Compound	*p*	*p* (Corr)	Formula	Composite Spectrum
4-Oxoproline	8E-04	0.023	C5 H7 N O3	(259.0921, 998.25)(130.04996, 50899.223)(131.0533, 3009.288)(132.05447, 0.0)
4-Nitrotoluene	1E-03	0.025	C7 H7 N O2	(138.05504, 99331.125)(139.05835, 6504.6655)(140.06024, 24.35)
Phenylpropiolic acid	3E-03	0.042	C9 H6 O2	(147.0439, 20949.69)(148.0471, 2231.3823)
C15 H21 N O7	2E-03	0.028		(328.1396, 55570.63)(329.14258, 9049.943)(330.1446, 1434.8273)(331.146, 0.0)
Tranylcypromine glucuronide	3E-03	0.045	C15 H19 N O6	(310.1289, 69001.52)(311.13202, 9635.0)(312.13416, 1680.972)(313.13696, 0.0)
Tryptophan	4E-04	0.014	C11 H12 N2 O2	(409.18713, 2513.0066)(410.1901, 963.9125)(205.09792, 142049.55)(206.10046, 14798.695)(207.1027, 365.41083)
C9 H7 N O	1E-04	0.010		(291.1072, 831.5)(146.06087, 127072.57)(147.0633, 10985.766)(148.06587, 16.289999)
Quinacetol	1E-03	0.023	C11 H9 N O2	(188.07137, 437216.25)(189.07404, 47215.27)(190.07632, 397.5903)(191.07889, 0.0)
Solanine + 5.759609	1E-04	0.010	C45 H73 N O15	(868.50684, 246811.27)(869.5104, 125611.68)(870.5127, 43195.08)(871.51514, 7611.9775)(872.518, 0.0)(873.51965, 0.0)
alpha-Chaconine	3E-04	0.014	C45 H73 N O14	(852.512, 303554.8)(853.50354, 142457.17)(854.5184, 42720.133)(855.5208, 6970.411)(856.52423, 0.0)(857.5257, 0.0)

Table 5.2 Fold Change analysis results of 'susceptibility only' interpretation in positive mode (Y = susceptible stock 23, N = non-susceptible stock 12) ('Experiment D').

Compound	Replication		Relative abundance normalized	
	Y (18)	N (23)	Y (18)	N (23)
4-Oxoproline	12	23	11.39653	17.59077
Phenylpropiolic acid	18	15	16.19037	10.37926
C15 H21 N O7	18	15	19.0777	11.93655
Tranylcypromine glucuronide	18	16	19.32866	12.8638
C9 H7 N O	18	23	19.27535	18.26517
Solanine + 5.759609	18	23	18.46194	20.48875
alpha-Chaconine	18	23	19.28908	20.99858

Table 5.3 Analysis of Variance (ANOVA) results of 'tissue vs. susceptibility' interpretation in positive mode ('Experiment D') ($P < 0.001$).

Compound	*p*	*p* (Corr)	Formula	Composite Spectrum
C16 H29 N O15	1E-04	2.97E-04		(476.1617, 18598.184)(477.1649, 3519.3352)(478.1662, 1069.6058)
CAY10492	5E-05	1.44E-04	C17 H22 N3 O2	(323.16068, 45571.785)(324.16373, 7654.3394)(325.16592, 1171.7334)
865.4838@4.639625	7E-06	4.64E-05		(866.4911, 45531.062)(867.49414, 22014.52)(868.49664, 5493.547)(869.50214, 1706.3833)
Solasonine	7E-10	6.98E-09	C45 H73 N O16]	(884.50183, 49257.707)(885.50494, 23117.084)(886.50714, 6394.738)(887.50977, 1381.625)
Solanine	8E-05	1.75E-04	C45 H73 N O15	(868.5071, 86457.5)(869.5101, 44123.547)(870.5127, 11530.425)(871.5147, 1769.5529)(872.5167, 0.0)
Nystatin A1	3E-05	1.44E-04	C47 H75 N O17	(926.5124, 71364.93)(927.51556, 34956.016)(928.51764, 12185.114)(929.51984, 3198.0)(930.5226, 0.0)
909.51@5.613134	5E-05	1.44E-04		(910.51746, 63347.195)(911.52075, 70139.68)(912.5229, 23405.545)(913.5251, 6124.279)(914.52655, 53.04)
C33 H53 N O6	7E-05	1.75E-04	C33 H53 N O6	(560.39557, 60087.08)(561.3987, 19864.34)(562.40137, 4487.647)(563.404, 1197.2571)
Solanidine	2E-04	3.00E-04	C27 H43 N O	(398.34222, 81160.39)(399.34564, 22225.172)(400.34857, 3082.23)(401.35156, 0.0)
Val Val	3E-16	6.93E-15	C10 H20 N2 O3	(217.15471, 21801.656)(218.15787, 2507.4128)
Phenylalanine	3E-05	1.44E-04	C9 H11 N O2	(166.08614, 19730.37)(167.08945, 2060.479)

Table 5.4 Fold Change analysis results of 'tissue vs. susceptibility' interpretation in positive mode (F = flesh, H = heart, Y = susceptible stock 23, N = non-susceptible stock 12) ('Experiment D').

Compound	Replication				Relative abundance normalized			
	FLESH N (12)	HEART Y (8)	FLESH N (12)	HEART Y (8)	FLESH N (12)	HEART Y (8)	FLESH N (12)	HEART Y (8)
C16 H29 N O15	12	3	2	0	16.518	6.127	2.843	0
CAY10492	12	3	0	0	16.921	6.135	0	0
865.4838@4.639625	12	2	2	0	17.420	4.299	2.888	0
Solasonine	12	1	0	0	17.228	2.294	0	0
Solanine	12	3	2	0	18.286	6.576	2.855	0
Nystatin A1	12	3	0	0	17.959	6.377	0	0
909.51@5.613134	12	3	0	0	18.059	6.510	0	0
C33 H53 N O6	12	3	3	0	17.500	6.316	4.420	0
Solanidine	12	4	4	0	18.527	8.667	6.04	0
Val Val	0	6	0	10	0	11.823	0	16.032
Phenylalanine	0	4	5	10	0	7.652	7.11	15.981

Appendix E)

1. Total ion chromatograms (TIC) of affected and control flesh and heart samples of stock 7 (susceptible to BH) and control samples of stock 3 (non-susceptible to BH) in negative and positive mode.

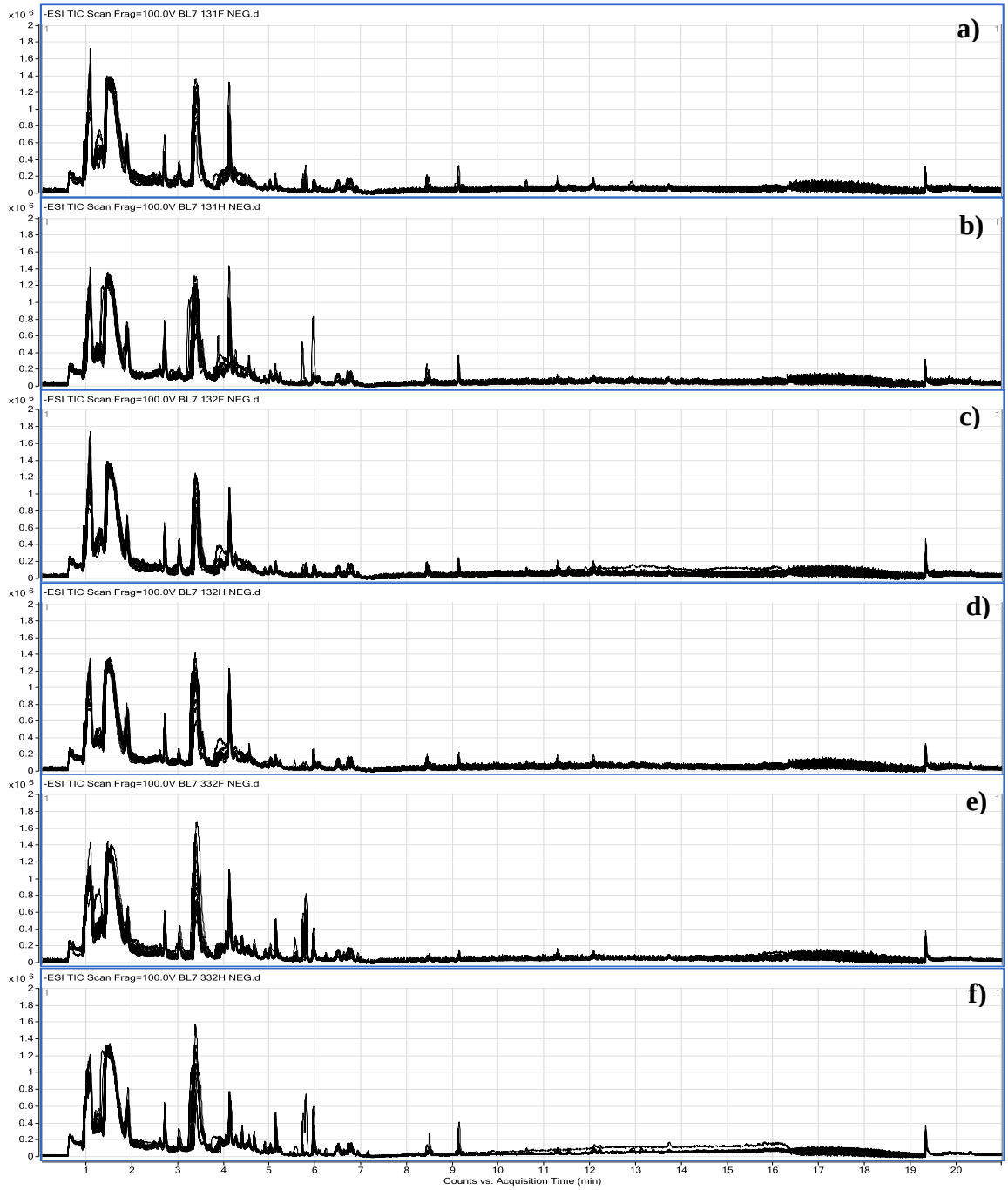

Figure 1.1 Total ion chromatograms of flesh and heart affected (a, b) and control (c, d) samples of stock 7 (susceptible to BH) and flesh and heart control samples (e, f) of stock 3 (non-susceptible to BH) in negative mode.

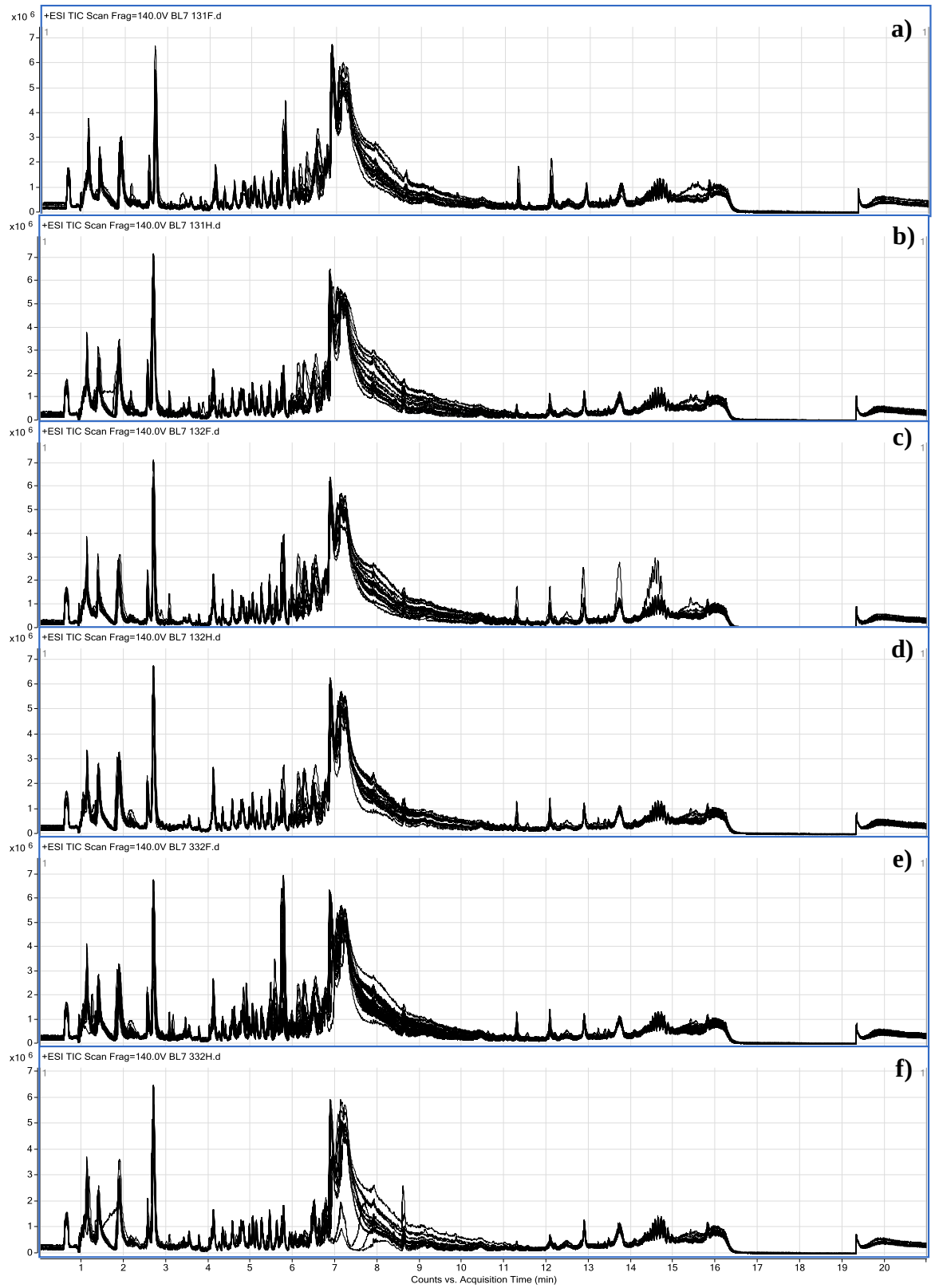

Figure 1.2 Total ion chromatograms of affected (a, b) and control (c, d) samples of stock 7 (susceptible to BH) and flesh and heart control samples (e, f) of stock 3 (non-susceptible to BH) in positive mode.

2. Metabolomic differences between affected and non-affected potato tuber tissues of one susceptible to BH stock (negative mode).

Table 2.1 Compounds identified in 'discoloration only' interpretation in negative mode ('Experiment E').

Compound	Formula	CompositeSpectrum
387.9471@0.6382856		(386.93982, 15564.555)(387.9412, 6973.12)(388.9387, 7569.059)(389.93945, 2339.2861)(390.9369, 1303.5454)
Ribose-1-arsenate	C5 H11 As O8	(272.95953, 57229.61)(273.96082, 15571.13)(274.95804, 15499.904)(275.95953, 2343.95)(276.95694, 875.32574)
C3 H4 N4 S2	C3 H4 N4 S2	(158.97913, 33913.594)(159.9805, 4997.8755)(160.97711, 4618.2827)
C13 H3 Cl N4 O6 S3	C13 H3 Cl N4 O6 S3	(440.8819, 5647.1465)(441.88324, 1932.605)(442.8806, 2663.449)(443.8819, 735.5868)(444.88092, 662.05005)
C11 H16 N6 O5	C11 H16 N6 O5	(311.11154, 41217.48)(312.11456, 4719.1855)(313.11652, 884.4451)
Asn His Gly	C12 H18 N6 O5	(325.12723, 51273.766)(326.1296, 7229.6704)(327.13205, 930.4863)
C18 H21 N9 O7	C18 H21 N9 O7	(474.1494, 146069.66)(475.1522, 25173.406)(476.1535, 5064.0864)(477.15512, 0.0)
Kojibiose	C12 H22 O11	(683.2271, 34174.773)(684.2305, 8459.652)(685.2325, 2331.052)(686.233, 511.54996)(341.11057, 25923.41)(342.114, 3374.0164)(343.11536, 911.3711)
Fructoselysine 6-phosphate	C12 H25 N2 O10 P	(387.11597, 56902.97)(388.1197, 7987.1304)(389.12122, 1642.9484)
C19 H30 N4 O14	C19 H30 N4 O14	(537.16864, 20164.615)(538.172, 3831.8284)(539.17346, 1125.0493)
L-Gulonate	C6 H12 O7	(195.0519, 52621.715)(196.0555, 3242.2856)(197.05663, 510.58163)
C18 H28 N4 O13	C18 H28 N4 O13	(507.15805, 29001.758)(508.1613, 4989.635)(509.16275, 1399.9191)
C16 H30 O16	C16 H30 O16	(477.14737, 26422.521)(478.15076, 4262.5605)(479.15225, 1468.2947)(480.1553, 0.0)
7-Methylxanthine	C6 H6 N4 O2	(165.0413, 67677.33)(166.04416, 3219.125)(167.04512, 287.787)
2-deoxy-ribonic acid	C5 H10 O5	(149.04578, 16621.572)(150.0467, 1296.0822)
His Ala Cys	C12 H19 N5 O4 S	(328.1074, 28922.467)(329.11023, 4106.7217)(330.10696, 1489.2107)
C21 H35 N O19	C21 H35 N O19	(604.17365, 19928.021)(605.177, 4379.546)(606.1792, 1260.6406)(607.1756, 743.3)
C9 H13 N O8	C9 H13 N O8	(525.1211, 1961.2521)(526.1248, 744.3)(262.05756, 18199.305)(263.06073, 1914.2507)(264.0624, 756.14)
2-Methylcitric acid	C7 H10 O7	(411.0881, 1399.6)(205.03612, 31050.607)(206.03947, 2325.6255)(207.04965, 813.6)
GW 9662	C13 H9 Cl N2 O3	(275.02234, 109689.6)(276.02585, 23064.967)(277.0292, 2800.3765)
4-Oxoproline	C5 H7 N O3,	(257.0782, 6499.3584)(258.0814, 1212.1559)(259.079, 517.7)(128.03593,

		23931.756)(129.03876, 1759.75)
5-(3-Pyridyl)-2-hydroxytetrahydrofuran	C9 H11 N O2	(164.07214, 21492.908)(165.07545, 2144.6357)
C18 H27 N5 O10 S2	C18 H27 N5 O10 S2	(536.11237, 10088.488)(537.115, 2280.526)(538.11127, 1319.5094)(539.1113, 583.9)
2-Methylcitric acid - 2.4877706	C7 H10 O7	(205.03586, 11770.304)(206.03925, 1047.4032)
Pantothenic Acid	C9 H17 N O5	(437.21368, 1420.2058)(438.21725, 954.2)(218.10388, 24805.248)(219.10721,2413.802) (220.10884, 548.26666)
C23 H17 N7 O8 S	C23 H17 N7 O8 S	(550.07904, 17958.91)(551.0818, 4855.4565)(552.0841, 1205.576)
Valiolone	C7 H12 O6	(191.05661, 62368.066)(192.06001, 4079.4736)(193.06123, 344.7316)
C18 H26 O17 S	C18 H26 O17 S	(545.082, 21191.064)(546.0849, 3927.1543)(547.082, 1934.8849)(548.08374, 486.92)
C18 H24 O13	C18 H24 O13	(895.23376, 3033.5833)(896.2375, 1439.3643)(897.2392, 1105.8501)(447.11472, 22519.006)(448.11786, 4020.566)(449.11942, 1305.943)
Kaempferol 3-[2''',3''',4'''-triacetyl-alpha-L-arabinopyranosyl-(1-6)-glucoside]	C32 H34 O18	(705.1663, 11562.3545)(706.1693, 3816.27)
C17 H16 N4 O9 S	C17 H16 N4 O9 S	(451.0553, 293680.0)(452.0588, 65944.79)(453.0572, 23305.863)(454.05902, 226.35716)(455.0583, 0.0)
Scopolin	C16 H18 O9	(707.182, 33615.965)(708.18536, 12135.868)(709.1876, 3126.65)(710.1895, 559.9176)(353.0882, 75665.234)(354.09167, 13297.273)(355.0954, 1742.0162)(356.10675, 365.3)
4-Methylumbelliferyl β-D-glucuronide	C16 H16 O9	(703.1495, 912.43756)(704.1533, 609.6)(351.07248, 10009.654)(352.07584, 1866.9458)
C16 H18 O13 S	C16 H18 O13 S	(449.03964, 32958.19)(450.04297, 5483.101)
Trp Asp Ile	C21 H28 N4 O6,	(431.1924, 12972.415)(432.19556, 2786.6)(433.1981, 755.14996)
Nap-HoPhe-OH	C28 H24 N2 O6	(483.15414, 40844.016)(484.1575, 7636.343)(485.15552, 2660.3125)(486.15698, 325.11765)
C18 H32 O13 S	C18 H32 O13 S	(487.14896, 5465.1133)(488.15204, 1195.7742)(489.14984, 737.91425)
C14 H18 O12 S	C14 H18 O12 S	(409.045, 25443.453)(410.0481, 3779.1978)(411.04532, 1510.5886)
C16 H23 N O13 S	C16 H23 N O13 S	(468.08188, 46885.35)(469.08514, 7594.176)(470.0824, 2849.7292)(471.0841, 134.25455)
Cefuroxime	C16 H16 N4 O8 S	(423.06064, 12249.204)(424.06384, 2107.1843)(425.0613, 891.4633)
C19 H24 O12	C19 H24 O12	(443.11945, 7974.0454)(444.1231, 1678.8823)(445.1247, 796.7143)
Glu Ala Trp	C19 H24 N4 O6	(807.32697, 795.4149)(403.16132, 17111.367)(404.16446, 3190.5562)(405.16666, 791.1023)
C20 H34 O11	C20 H34 O11	(449.20316, 6849.731)(450.20627, 1520.746)(451.20822, 612.19995)

Glu Trp Pro	C21 H26 N4 O6	(429.17673, 7755.5176)(430.18005, 1740.4954)(431.1822, 642.2)
6-Hydroxymellein	C10 H10 O4	(193.05109, 26987.35)(194.05443, 2680.7302)(195.05641, 411.58334)
C14 H14 O8	C14 H14 O8	(619.1292, 3760.079)(620.1323, 3809.5203)(621.1367, 3330.2)(622.1393, 0.0)(309.06207, 25988.674)(310.06537, 3613.224)(311.067, 958.2214)
Glu Trp Pro - 5.998713	C21 H26 N4 O6	(859.35803, 1096.9827)(860.3605, 768.5571)(429.17688, 24869.89)(430.18002, 4966.2695)(431.18234, 1085.4528)
Flucarbazone	C12 H11 F3 N4 O6 S	(395.02948, 18003.908)(396.03247, 2606.2146)(397.02972, 1368.5192)
Methyl N-(a-methylbutyryl)glycine	C9 H16 O4	(187.09793, 20720.986)(188.10135, 2025.2252)(189.1035, 117.0)
1510.2983@6.4970164		(1509.2908, 21383.928)(1510.2921, 11014.467)(1511.2919, 1708.724)
1509.9633@6.4970317		(1508.9568, 15211.71)(1509.9589, 16774.785)(1510.9597, 3304.8062)(1511.9575, 583.7)
1510.6324@6.497031		(1509.625, 21317.07)(1510.6257, 6339.8237)(1511.6257, 899.0263)
1453.9562@6.6183043		(1452.9502, 7458.0894)(1453.9504, 3681.2874)(1454.9493, 801.05707)
1424.6143@6.708936		(1423.6067, 22530.215)(1424.6085, 10213.934)(1425.6077, 1504.9357)
1424.2784@6.7088885		(1423.2731, 16650.44)(1424.274, 16044.914)(1425.2745, 2927.737)
1424.9481@6.7086506		(1423.9406, 21361.97)(1424.9408, 5703.121)(1425.9403, 793.65564)
1434.617@6.776508		(1433.6095, 18200.584)(1434.6107, 18424.023)(1435.611, 3442.8665)(1436.6064, 686.2)
1434.951@6.7764907		(1433.9438, 24913.584)(1434.9453, 11783.872)(1435.9447, 1729.88)
1435.2842@6.7764115		(1434.2778, 24073.521)(1435.2782, 6647.4194)(1436.2759, 890.5099)
9S,10S,11R-trihydroxy-12Z-octadecenoic acid	C18 H34 O5,	(659.4718, 1247.1234)(660.4752, 718.975)(329.23297, 53012.42)(330.23636, 11266.499)(331.23877, 1218.0367)
9-hydroperoxy-12,13-epoxy-10-octadecenoic acid	C18 H32 O5	(327.21722, 28997.713)(328.22064, 5238.8296)
9S,10S,11R-trihydroxy-12Z-octadecenoic acid - 9.125128	C18 H34 O5	(659.4715, 876.26666)(329.23276, 66572.4)(330.2362, 11848.26)(331.23865, 1150.9945)
LysoPE(18:3(9Z,12Z,15Z)/0:0)	C23 H42 N O7 P	(949.5287, 926.19995)(950.5329, 540.4)(474.26202, 11678.398)(475.26532, 2781.3525)(476.26758, 897.77386)
C22 H45 N9 O6 S	C22 H45 N9 O6 S	(562.3143, 26579.93)(563.31744, 7044.612)(564.3195, 1433.0638)(565.32166, 0.0)
PE(18:2(9Z,12Z)/0:0)	C23 H44 N O7 P	(953.55994, 710.25)(476.27798, 11374.428)(477.28113, 2767.8508)(478.28354, 786.5731)
C22 H47 N9 O6 S	C22 H47 N9 O6 S	(564.3299, 22074.174)(565.3331, 5918.2017)(566.33545, 1333.1189)(567.33765, 0.0)
C9 H Cl N2 O11 S2	C9 H Cl N2 O11 S2	(410.8628, 6275.678)(411.86438, 1331.613)(412.8612, 2445.9597)(413.8609, 590.0)

3. Metabolomic differences between control samples of potato stocks with different susceptibility to blackheart disorder (negative mode).

Table 3.1 Analysis of Variance (ANOVA) results of 'susceptibility only' interpretation in negative mode ('Experiment G') ($P < 0.001$).

Compound	*p*	*p* (Corr)	Formula	Composite Spectrum
870.1731@4.3968663	1E-05	2.06E-04		(869.1653, 36800.574)(870.1683, 11924.942)(871.1682, 4527.8574)(872.16943, 1385.0076)(873.17096, 0.0)
Quercetin 3-glucoside-7-rutinoside	3E-10	3.07E-08	C33 H40 O21	(771.1979, 11885.306)(772.20123, 3980.8022)(773.20306, 1356.3169)(774.20416, 662.85)
C27 H32 O21 S	5E-05	4.50E-04		(723.10767, 24387.309)(724.1108, 6624.4297)(725.1099, 2635.1301)(726.1116, 923.9094)
Myricetin 3-rutinoside	2E-05	2.65E-04	C27 H30 O17	(1251.2856, 1282.9667)(1252.2913, 862.65)(625.1404, 14345.914)(626.1435, 3881.6343)(627.14557, 1349.1915)(628.14795, 421.7)
C19 H24 O12	9E-06	1.70E-04		(887.24414, 1611.75)(888.2488, 1062.6)(443.1196, 10359.922)(444.12308, 2092.2031)(445.12503, 897.1429)
C20 H32 N6 O18 S2	3E-05	2.65E-04		(707.1133, 11973.106)(708.11615, 3458.268)(709.11334, 1693.5717)(710.1128, 765.6125)
Quercetin 3-glucoside-7-rhamnoside	2E-05	2.65E-04	C27 H30 O16	(1219.2963, 8643.199)(1220.2997, 5237.828)(1221.3021, 2668.2498)(1222.304, 1088.63)(609.1459, 63524.348)(610.149, 16304.978)(611.1512, 3856.0947)(612.15356, 267.98148)
Imibenconazole	2E-08	5.07E-07	C17 H13 Cl3 N4 S	(408.98718, 10784.114)(409.98914, 2709.7292)(410.98898, 1160.3057)
C14 H14 O8	2E-08	5.07E-07		(619.12946, 4808.7275)(620.1329, 1981.3378)(621.13513, 1213.9501)(309.0621, 40430.21)(310.0654, 5454.6997)(311.06717, 1056.0311)
Isoferulic acid	4E-08	8.40E-07	C10 H10 O4	(193.05104, 40955.973)(194.05446, 3922.8147)(195.05637, 308.84613)
9S,10S,11R-trihydroxy-12Z-octadecenoic acid	4E-05	3.26E-04	C18 H34 O5	(659.47174, 1063.6454)(660.475, 622.25)(329.23306, 40934.703)(330.23648, 8680.717)(331.23883, 1073.7)

Table 3.2 Fold Change analysis results of 'susceptibility only' interpretation in negative mode (Y = susceptible stock, N = non-susceptible stock 3) ('Experiment G').

Compound	Replication		Relative abundance normalized	
	Y (31)	N (32)	Y (31)	N (32)
870.1731@4.3968663	28	32	13.459	17.364
Quercetin 3-glucoside-7-rutinoside	14	32	6.182	15.278
C27 H32 O21 S	29	32	13.744	16.694
Myricetin 3-rutinoside	26	32	11.583	15.807
C19 H24 O12	31	32	14.036	15.074
C20 H32 N6 O18 S2	24	32	10.823	15.642
Quercetin 3-glucoside-7-rhamnoside	29	32	14.755	18.218
Imibenconazole	16	32	7.068	15.004
C14 H14 O8	31	32	15.824	17.501
Isoferulic acid	31	32	15.870	17.422
9S,10S,11R-trihydroxy-12Z-octadecenoic acid	31	32	17.509	16.450

4. Metabolomic differences between control samples of potato stocks with different susceptibility to blackheart disorder (positive mode).

Table 4.1 Analysis of Variance (ANOVA) results of 'susceptibility only' interpretation in positive mode ('Experiment H') ($P < 0.05$).

Compound	*p*	*p* (Corr)	Formula	Composite Spectrum
Monoethylglycylxylidide (MEGX)	8E-04	0.024	C12 H18 N2 O	(207.14952, 47441.316)(208.15288, 5961.3975)(209.1556, 137.42632)
Quercetin 3-glucoside-7-rutinoside	3E-10	0.000	C33 H40 O21	(773.2143, 68843.01)(774.2175, 22772.312)(775.21954, 6067.345)(776.2216, 989.07043)(777.2256, 0.0)
Leu Ile Ile	9E-04	0.027	C18 H35 N3 O4	(358.27072, 42817.34)(359.2737, 8199.884)(360.27615, 1103.5652)
Myricetin 3-rutinoside	5E-10	0.000	C27 H30 O17	(627.1565, 36697.97)(628.15955, 10055.703)(629.16174, 2602.893)(630.1646, 447.11255)
3,5,7,2',5'-Pentahydroxyflavone	1E-06	0.000	C15 H10 O7	(303.0505, 55786.098)(304.0538, 8152.9497)(305.0566, 1236.2909)
Phe Phe Val	7E-04	0.024	C23 H29 N3 O4	(823.4401, 770.6)(412.22394, 40646.67)(413.22684, 9985.532)(414.2296, 1593.0985)(415.2404, 1138.4)
3,5,7,2',5'-Pentahydroxyflavone + 5.129875	4E-08	0.000	C15 H10 O7	(303.05057, 52475.53)(304.05383, 8039.1216)(305.05576, 1222.9784)
Rutin	1E-05	0.001	C27 H30 O16	(611.16174, 86573.84)(612.1651, 23583.197)(613.1671, 5307.6416)(614.16986, 532.8061)
1064.669@6.306651	1E-03	0.039		(533.3415, 20631.629)(533.84326, 10855.016)(534.3445, 3949.0896)(534.84534, 1159.2445)
2229.934@6.4383016	2E-05	0.001		(1115.9751, 88824.91)(1116.4761, 65831.03)(1116.9766, 27919.0)(1117.4763, 10634.813)(1117.9728, 3252.9563)(1118.464, 493.49994)
2229.4333@6.438286	2E-05	0.001		(1115.7245, 72292.875)(1116.2256, 88015.23)(1116.7262, 48867.227)(1117.2264, 17540.594)(1117.7258, 7123.9775)(1118.2202, 1269.1063)(1118.7139, 1156.85)

Table 4.2 Fold Change analysis results of 'susceptibility only' interpretation in positive mode (Y = susceptible stock 7, N = non-susceptible stock 3 ('Experiment H').

Compound	Replication		Relative abundance normalized	
	Y (31)	N (32)	Y (31)	N (32)
Quercetin 3-glucoside-7-rutinoside	13	32	6.842	17.722
Myricetin 3-rutinoside	13	32	6.609	16.873
3,5,7,2',5'-Pentahydroxyflavone	19	32	9.855	17.482
3,5,7,2',5'-Pentahydroxyflavone + 5.129875	16	32	8.279	17.320
Rutin (or quercetin-3-O-rutinoside)	22	32	11.787	18.290