Final Report

Blackheart - an emerging problem for the GB potato packing industry

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1. SUMMARY

The aim of this component of the project was to study the physiological and biochemical mechanisms involved in blackheart (BH) disorder. The number of tubers (from a stock) which can be studied using the detailed biochemical and physiological techniques required is limited and as such has often meant that in the absence of a method to reliably induce blackheart in 100% of tubers of a susceptible stock, the researchers have had to work with tubers that never developed blackheart symptoms. As a result, it has been difficult to draw firm conclusions about the factors which contribute to, or are characteristic of, blackheart symptom expression. Based on the information that is available the conclusions are:

- Cold initial temperature (1.5^{o}C) was the main factor influencing both respiration rate and compositional changes in potato tubers from stocks with different susceptibility to BH.
- A relation between respiration rate and BH could not be made.
- The temperature and exposure period in which BH shows greater incidence could not readily be predicted. It is still unclear whether brown tissue discolorations seen 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.
- Similarities in biochemical changes between susceptible and non-susceptible BH stocks were observed in both years 1 and 2.
- Sugars tended to be more accumulated in the BH susceptible stocks compared to the non-susceptible stocks.
- Chlorogenic acid was highly accumulated in both control and discoloured samples of susceptible to BH stocks.
- Untargeted metabolomics lead to the identification of differences in chemical composition between samples of tissues with blackheart symptoms and those without (and between tubers from stocks designated as BH susceptible and those designated as non-susceptible). The differences were more pronounced in year 1 of the work. A larger number of samples were analysed in year 2 of the study and further biochemical research is needed in order to understand how differences in the composition/quantity of metabolites between tissue samples are related to BH susceptibility.

2. INTRODUCTION

The aim of this component of the project was to study the physiological and biochemical mechanisms involved in blackheart (BH) disorder. The SBCSR induction method (see Report No. 2015/9) was used to select potato stocks (cv. Maris Piper) with different susceptibility to BH. A range of techniques were applied to samples of the selected stocks in three seasons of experiments. The methods and results of these experiments are summarized below.

2.1. Effect of storage temperature and shelf-life conditions on BH incidence

According to customer complaints, BH disorder in potato tubers is more evident by the end of the winter, peaking in spring time. One hypothesis is that inappropriate storage and/or shelf-life conditions may contribute to BH development. In this experiment, Maris Piper stocks were exposed to different storage temperatures and monitored (respiration rate, biochemical analysis) before and after transfer to different shelf-life conditions.

Methods

Year 1 (2011-2012)

Three potato stocks with different susceptibility to BH were initially stored at 1.5 or 3^{o}C and then transferred to 15^{o}C for shelf-life evaluation in air and/or CA. In total there were seven sampling points (outturns). Samples of tubers, including flesh, heart and peel tissues, were taken at the outturns and stored (frozen) pending biochemical analysis.

Year 2 (2012-2013)

Three potato stocks with different susceptibility to BH were initially stored at 1.5^{o}C until required. The experiment ran from 7/12/2012 to 10/05/2013. Tubers were transferred to 20^{o}C in air and sampled on two sampling days post transfer. In total there was a baseline and five sampling points (outturns). Samples of tubers, including flesh, heart and peel tissues, were taken at the outturns and stored (frozen) pending biochemical analysis.

Additionally, some tubers were stored at 1.5^{o}C and then transferred (19th June 2013) to 20^{o}C under various gas combinations. The experiment lasted 14 days (ending on the 3rd July 2013).

Note on the methods:

It is 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). Therefore, 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 allowed before sampling. However, during this project condensation was not observed on the tubers.

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. Two potato stocks cv. Maris Piper (one susceptible and one non-susceptible to BH) were studied. The microstructure and O_2 diffusivity of potato tuber tissue samples (flesh and heart) were recorded in order to understand if there is a detectable relationship with BH incidence.

Results

Blackheart symptoms:

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, depending on the severity (Hooker, 1981). It is reported 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). In this project, less and more intense brown tissue discolorations localized in tuber pith were mostly identified and sometimes accompanied with brown scattered blotches (Fig. 1).

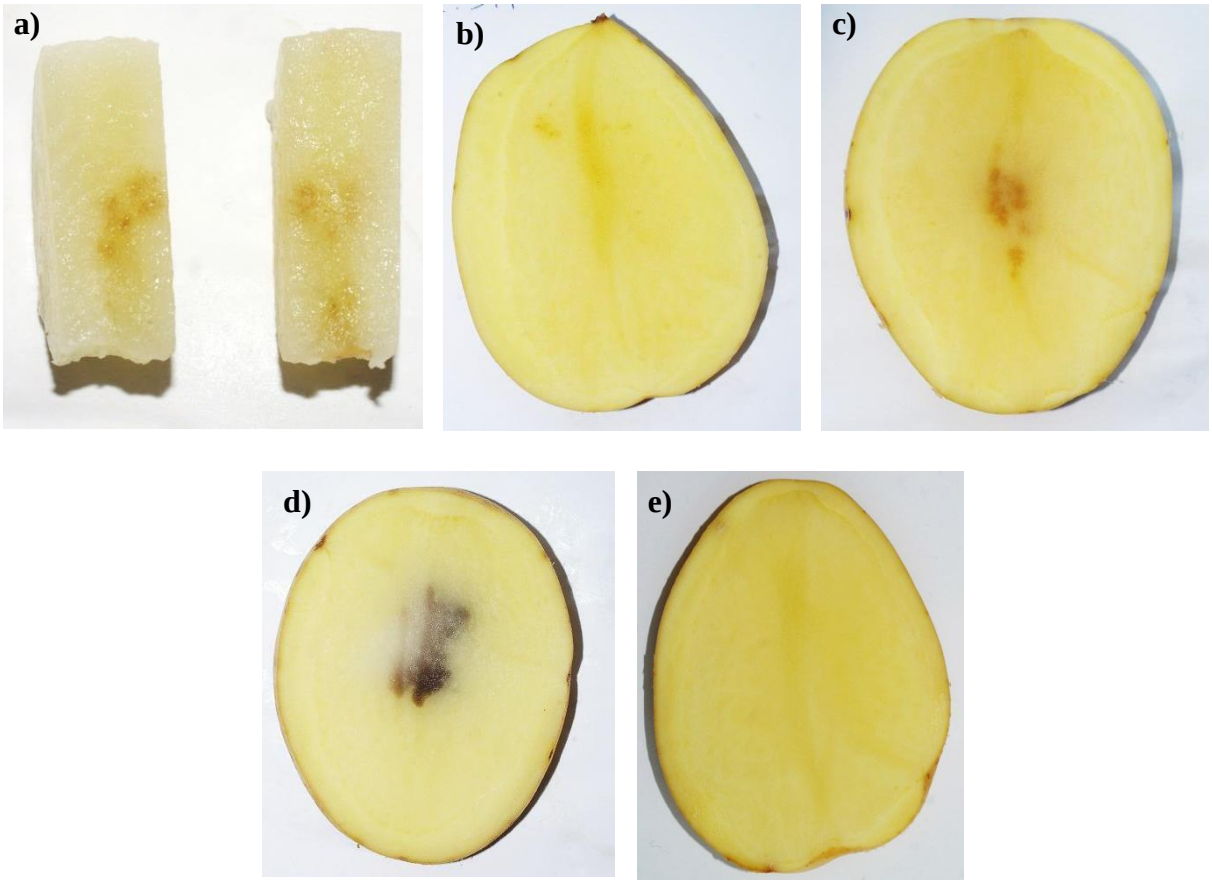

Figure 1 Examples of affected potato tubers cv. Maris Piper indicating tuber tissue discoloration as brown centre light (BCL) (a), pith (b), brown centre (BC), dark brown to black (BH) (d). Control tuber showing no discoloration (e).

Year 1 & Year 2

In year 1, brown tissue discolorations localized in the central pith part of the tuber occurred in the two stocks which had been designated BH-susceptible (on the basis of the initial evaluation using the SBCSR induction method). BH in these stocks (stock 20 and stock 23) was first observed in December 2012 after 8 weeks of storage at low temperature (1.5 or 3^{o}C), peaking after a few months during springtime. However, the total percentage of BH incidence was low (< 10%). Stock 23 had significantly (ca. 3-fold) greater BH incidence compared to stock 20 and yet BH-like symptoms were found in just three tubers of stock 23 (out of 504 tested). No indications of discoloration were observed at the baseline sampling (November 2011), when no initial cold storage temperature had yet occurred. This might suggest that the cold storage at low temperature (1.5 or 3^{o}C), after baseline may trigger the brown tissue discoloration.

In year 2, the incidence of BH in both experiments conducted was so low that no conclusions on the impact of storage and/or shelf life conditions could be drawn.

Respiration rate

Year 1

There was an increase in respiration rate on sampling day 3 and then a slight decrease on sampling day 7. 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^oC compared with those at 3^oC and this was more pronounced in CA conditions. However, there was a variation in respiration rate between the stocks analysed over storage time (Fig. 2 and 3).

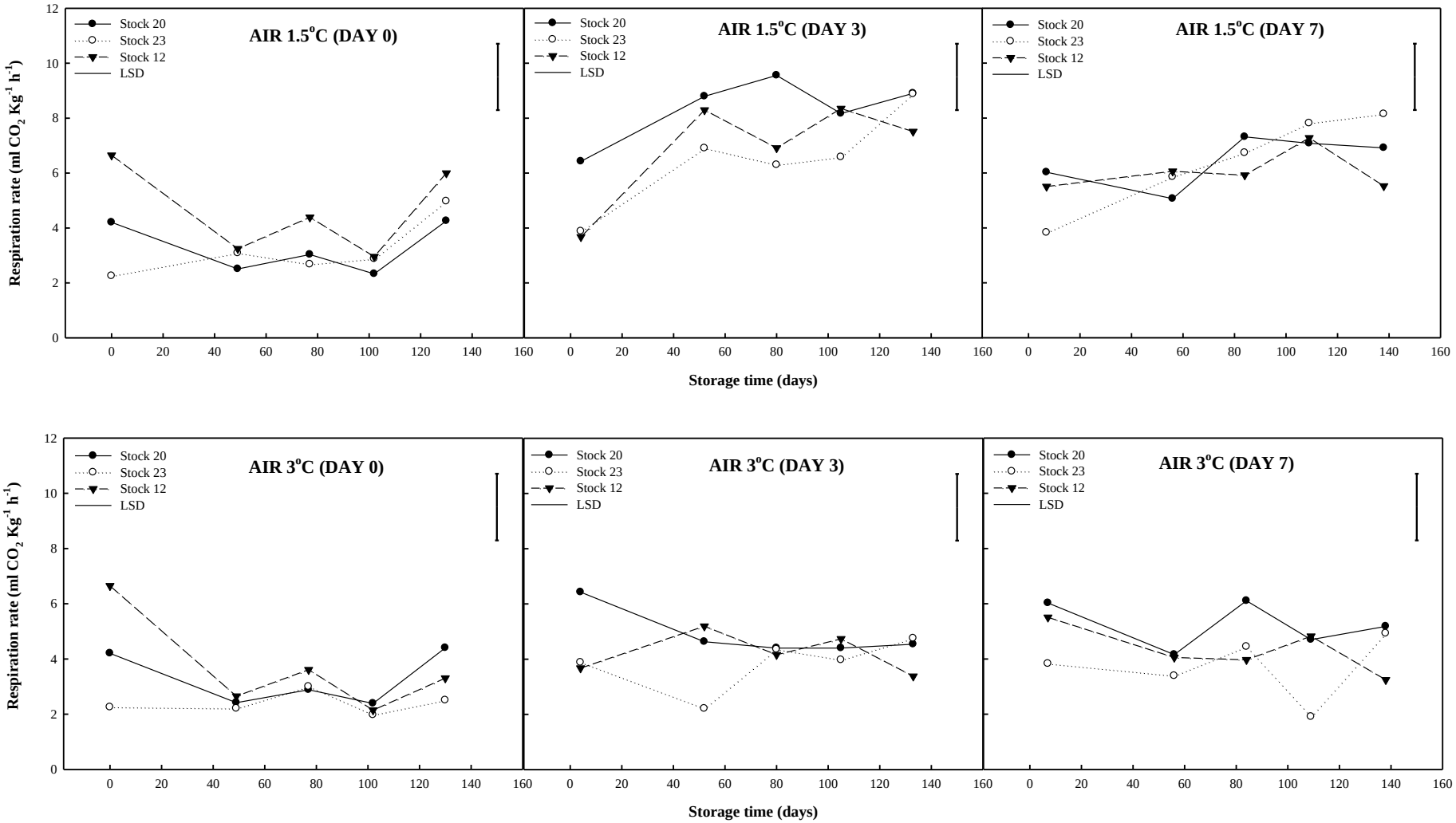

Figure 2 Respiration rate (ml CO_2 kg^{-1} h^{-1}) of stock 20 (susceptible to BH), stock 23 (susceptible to BH) and stock 12 (non- susceptible to BH) recorded over storage time in air only at 15ºC on sampling days 0, 3 and 7. Initial storage temperature and general LSD is shown.

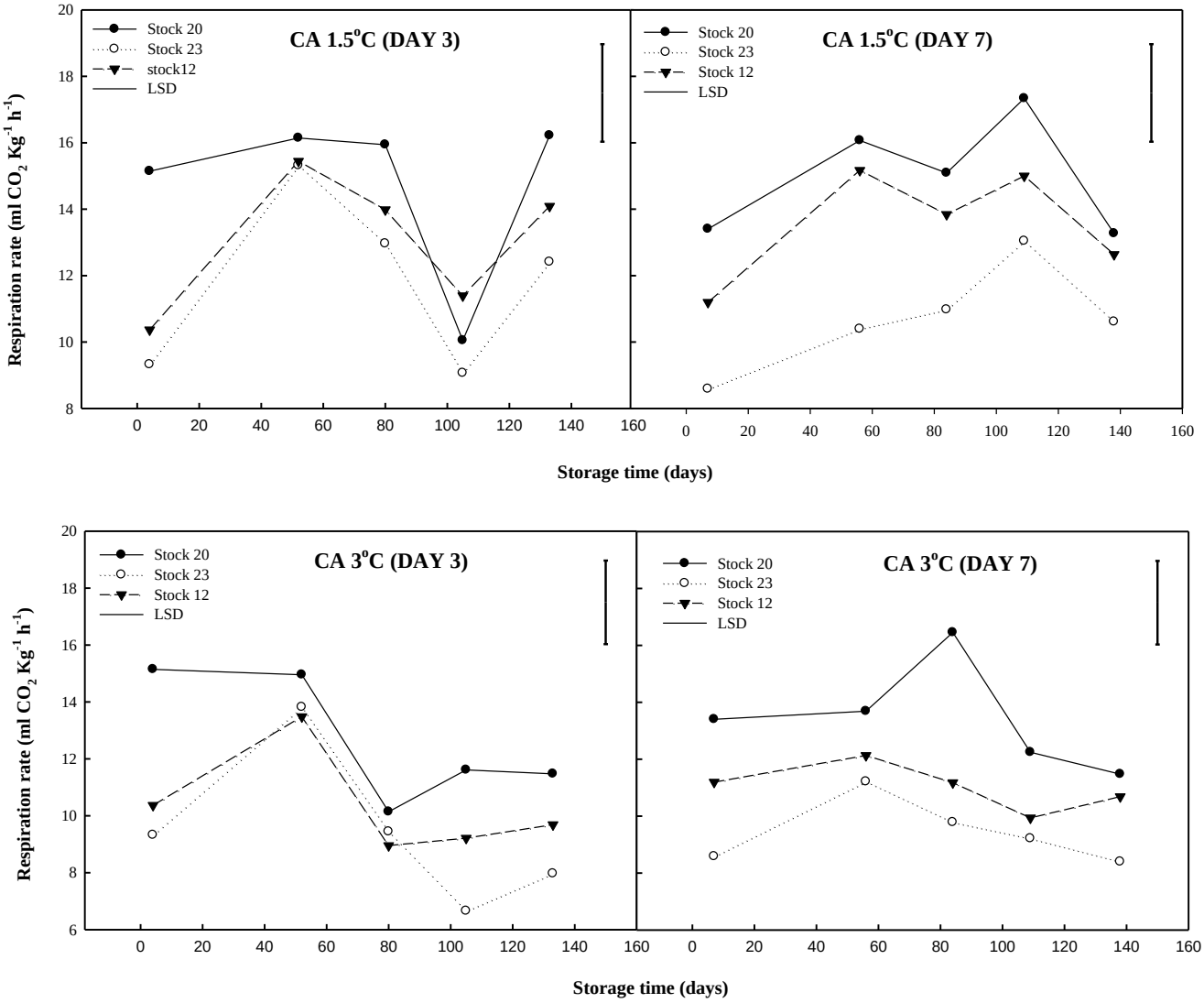

Figure 3 Respiration rate (ml CO_2 kg^{-1} h^{-1}) of stock 20 (susceptible to BH), stock 23 (susceptible to BH) and stock 12 (non- susceptible to BH) recorded after storage in CA (10% CO_2) at 15ºC on sampling days 3 and 7 over storage time. Initial storage temperature and general LSD is shown.

Year 2

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) (Fig. 4). Because the incidence of BH was so low, it was not possible to examine if there was any statistically significant relationship between BH susceptibility and respiration rate.

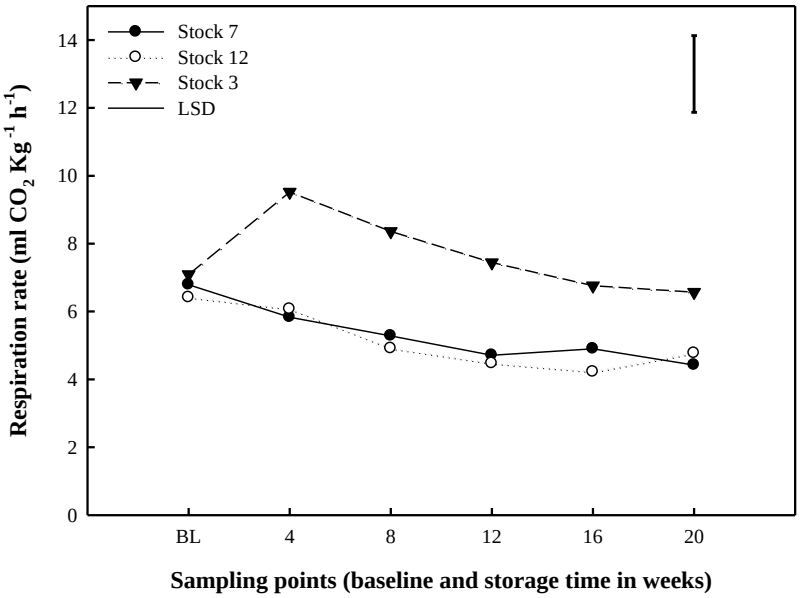

Figure 4 Respiration rate (ml CO_2 kg^{-1} h^{-1}) of potato stocks cv. Maris Piper [stock 7 (susceptible to BH), stock 12 (susceptible to BH) and stock 3 (non-susceptible to BH)] recorded after baseline (BL) and storage in air at 20°C. Tubers were initially stored at 1.5°C for 4, 8, 12, 16 and 20 weeks.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

Overall, according to the findings in both years it seems that differences in respiration rate were due to low initial storage temperature. The studies of different post transfer atmospheres (Year 1: viz. 21% O_2; 10% CO_2; 10% O_2 and 5% O_2 [data not shown]) indicated that respiration rate was lower at 5% O_2, but this did not result in a significantly different incidence of BH, compared to the other atmospheres studied.

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

Years 1 & 2

The tuber size (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 similar 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 the dimensions measured, nor the weight of tubers, had any observable effect on BH incidence.

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). The incidence of BH was so low that no conclusions on the impact of tuber size on BH could be drawn.

2.3. Gas diffusivity measurements

Year 3

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 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). 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 ultimately lead to physiological changes and tissue cell impairment and cell death (Geigenberger *et al.*, 2000; Ferreira de Souza *et al.*, 2002; Verboven *et al.*, 2008; Zabalza *et al.*, 2009). As the gas exchange mechanism in tubers is largely dependent on the intercellular space and cell structure arrangement, X-ray micro-computed tomography was used in year 3 to study tuber tissue. The technique allows the tissue structure in flesh and heart tissue samples to be visualized. The samples were taken from tubers of two Maris Piper stocks which had been designated either BH-susceptible[stock 10] or BH non-susceptible [stock 4] on the basis of an initial evaluation using the SBCSR induction method. Oxygen diffusivity and respiration rate were also measured for the samples. Because of the nature of the methods it was only possible to study tissue samples from two tubers of each stock for the X-ray CT work and eight tubers per stock for the O_2 diffusivity work.

Results

Both micro-CT scans (Fig. 5) and 3D images (Fig. 6 and 7) showed that the gas-filled intercellular spaces of flesh and heart tissue were not well connected and varied in size and shape.

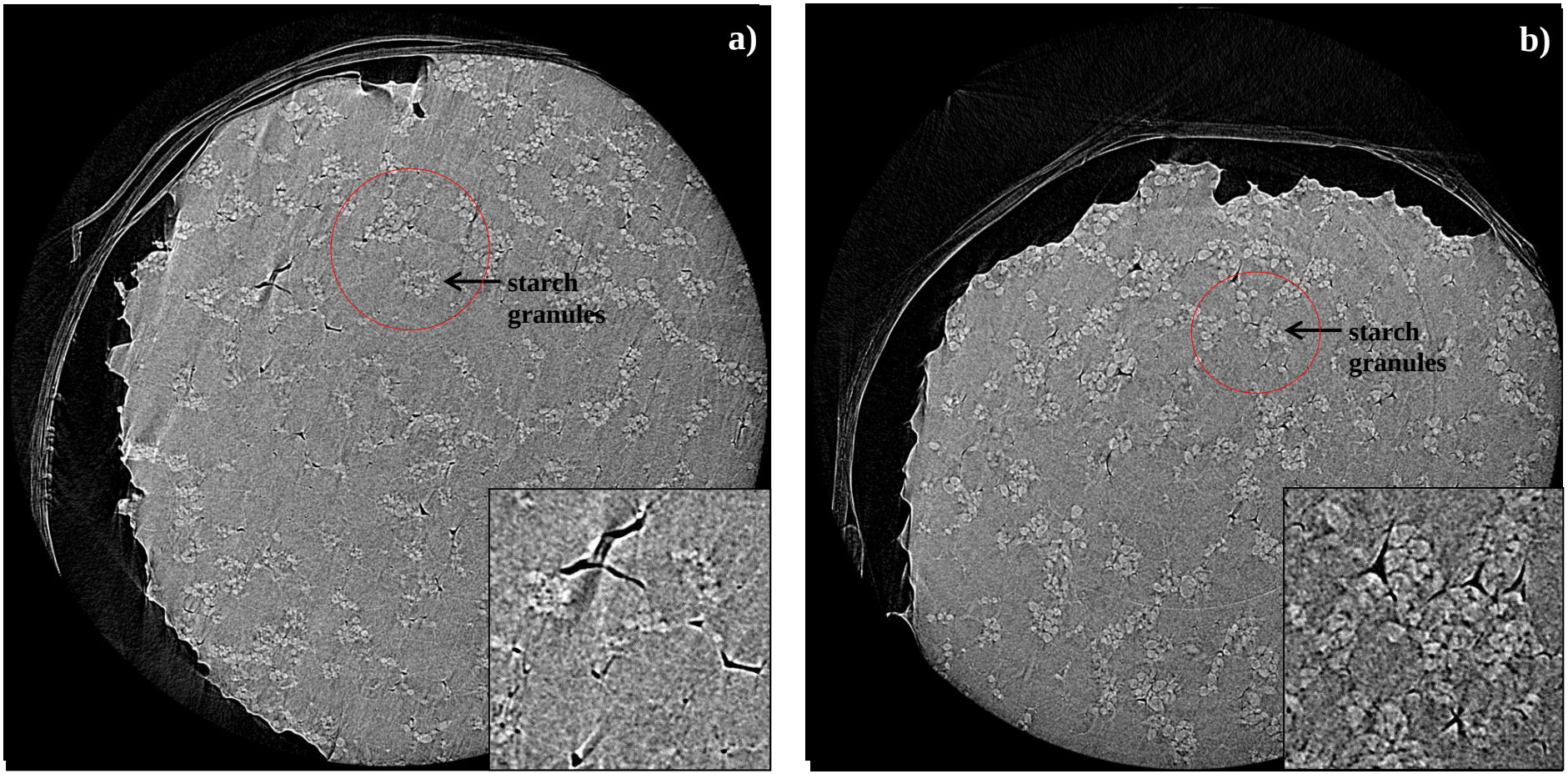

Figure 5 X-ray micro-CT scans of flesh (a) and heart (b) tissue (3 mm in diameter and 5 mm in thickness) of tuber 1 from stock 4 (non-susceptible to BH) at 2 µm pixel resolution. Intercellular spaces are shown as a black hole shape. Suspected starch granules are shown in light grey colour. Selected regions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

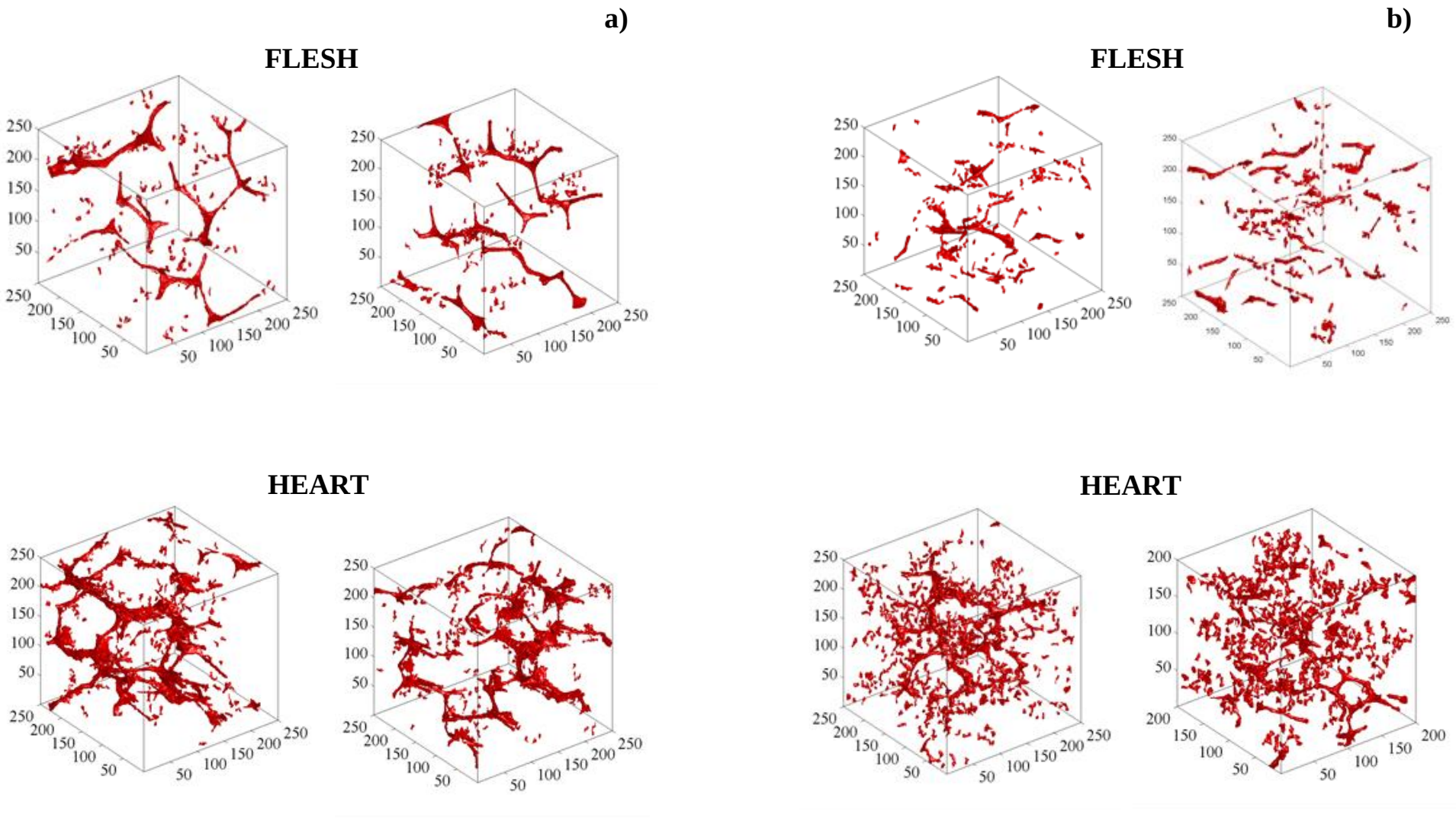

Figure 6 3D microstructure of flesh and heart tissue of tuber 1 (a) and tuber 2 (b) from stock 4 (non-susceptible to BH) reconstructed after micro-CT scanning.

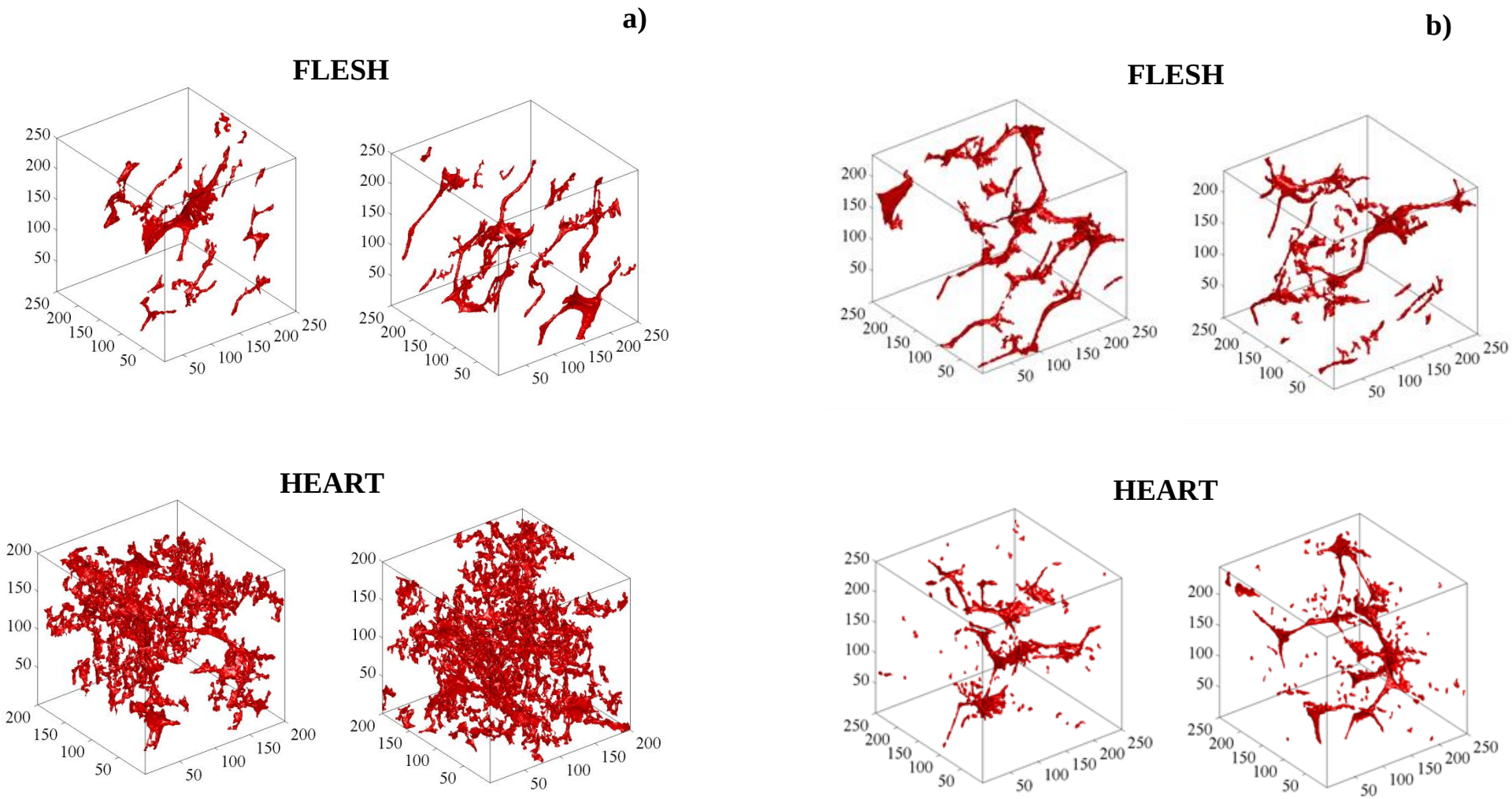

Figure 7 3D microstructure of flesh and heart tissue of tuber 1 (a) and tuber 2 (b) from stock 10 (susceptible to BH) reconstructed after micro-CT scanning.

During the experiment all tuber tissue samples were sound with no indications of discoloration except a brown centre (BC) discoloration that occurred in a tuber of stock 4 (non-susceptible to BH) at measurement on day 7 (Fig. 8).

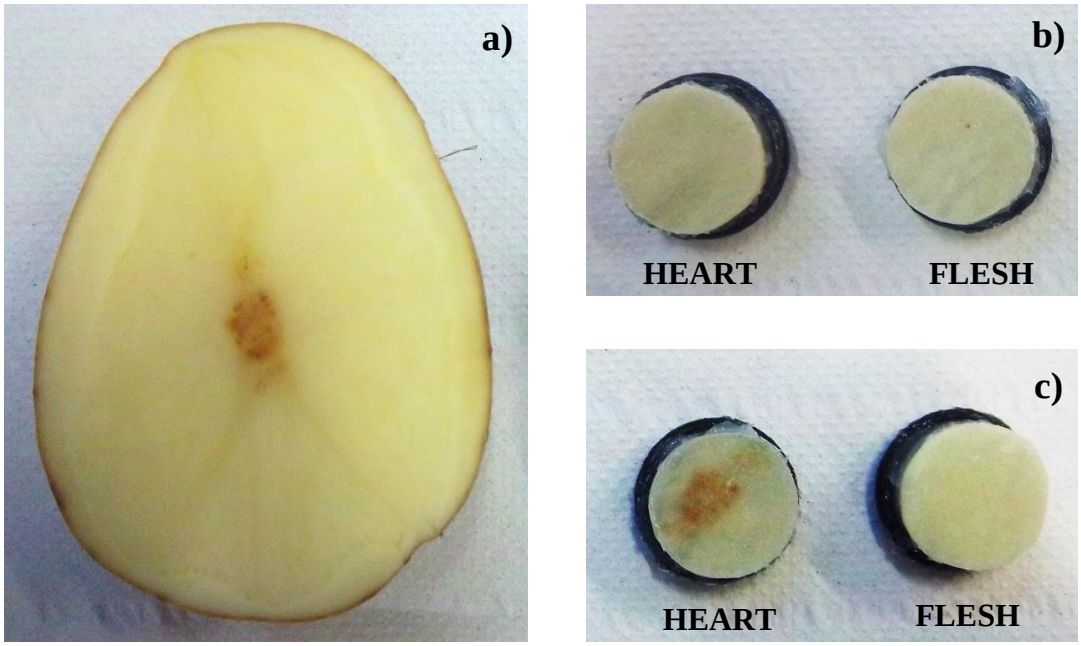

Figure 8. BC (brown center) discoloration in tuber of stock 4 (non-susceptible to BH) (a). Heart and flesh tissue samples of stock 10 (susceptible to BH) (b) and stock 4 (c) before O_2 diffusion measurement on day 7.

According to Table 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 1).

Table 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}

Because the tubers of the selected stocks didn't clearly show consistent differences in the incidence of BH during the period of study it was not possible to draw conclusions as to whether there was a significant relationship between either the size and/or shape of the intercellular spaces in the tuber tissue and BH nor between the measured rates of O_2 diffusivity and BH incidence.

Variability in the organisation of intercellular spaces was observed between flesh and heart tuber tissue. This was due to "noise" caused by starch accumulation and also the sampling position of the potato tubers from which flesh and heart tissues were cut and scanned.

The technique to study O_2 diffusivity has been extensively used to elucidate changes in gas diffusivities of pear fruit with or without core breakdown disorder (Ho *et al.*, 2006a, b 2007). The results reported here indicate that further studies using the technique would be warranted as there is currently a dearth of information on the gas diffusivity of different regions of the potato tuber. Further studies would provide a larger dataset and help understand whether BH is induced when O_2 is depleted and consumed faster than it may be supplied resulting in cell necrosis mainly in the central part of the tuber (as proposed by Lipton, 1967; Smith, 1978; Banks and Kays; 1988). It would be particularly interesting to estimate the O_2 diffusion in specific regions of the perimedullary and the central pith.

2.4. Biochemical changes: non-structural carbohydrates

Years 1 & 2

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 samples of tubers that were initially stored at 1.5^{o}C.

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 rapid sucrose degradation to reducing sugar (fructose, glucose) 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).

In year 1, although there was an increase through storage time, both sucrose and reducing sugar content varied among sampling days 0, 3 and 7. On Day 0 (Baseline), all the tubers were held at 15^{o}C. After this time point tubers were transferred to 1.5^{o}C. The baseline measurements did not show significant differences in fructose, glucose or sucrose content between the three stocks or between heart and flesh tissue samples. After the transfer to 1.5^{o}C, stock 23 (susceptible to BH) accumulated higher levels of fructose and glucose compared to the other two stocks. After 8 weeks of storage, stock 23 had significantly higher sucrose content compared to the other two stocks. In general, it was observed that higher sugar content was accumulated more in the heart when compared to flesh tissue samples. In year 1, brown tissue discolorations were mostly observed 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 suggested that the whole tuber was affected after storage at low cold temperature (Fig. 9).

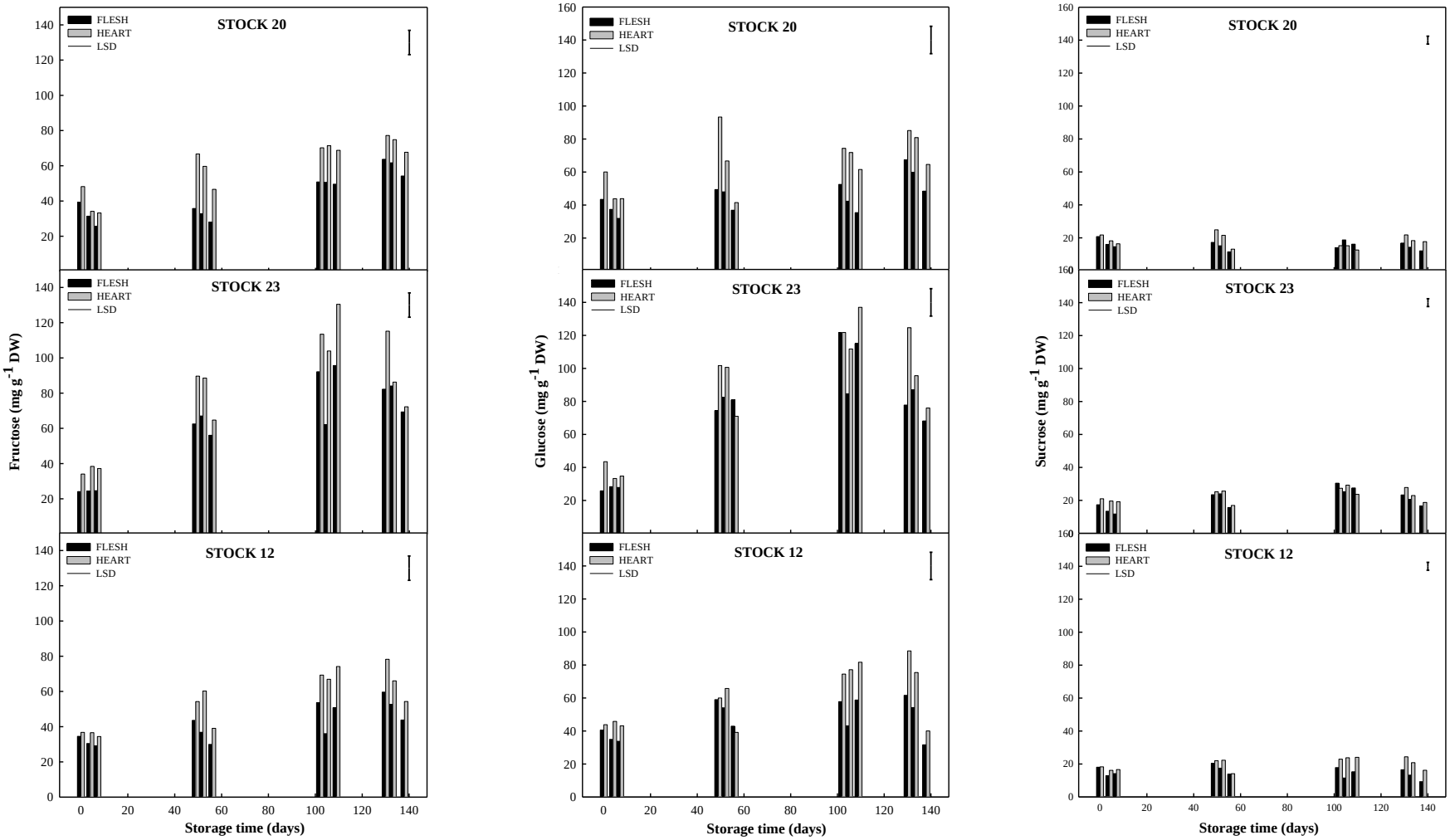

Figure 9 Fructose (a), glucose (b) and sucrose (c) concentrations (mg g^{-1} DW) in flesh and heart tissue of potato tubers Maris Piper stock 20 (susceptible to BH), stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) at baseline and after storage in air only at 15^{o}C on sampling days 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 ($P < 0.05$).

In year 2, on day 0 (Baseline, BL), all the tubers were held at 20^{o}C. After this date tubers were transferred to 1.5^{o}C. Fructose and glucose 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 significantly during storage time. Sucrose content of stock 3 (non-susceptible to BH) was significantly lower compared to stock 7 (susceptible to BH). 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. In year 2, the incidence of BH symptoms was lower than in Year 1. Stock 7 included tubers which developed BH symptoms. 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 (Fig. 10). 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. 11). 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. 12).

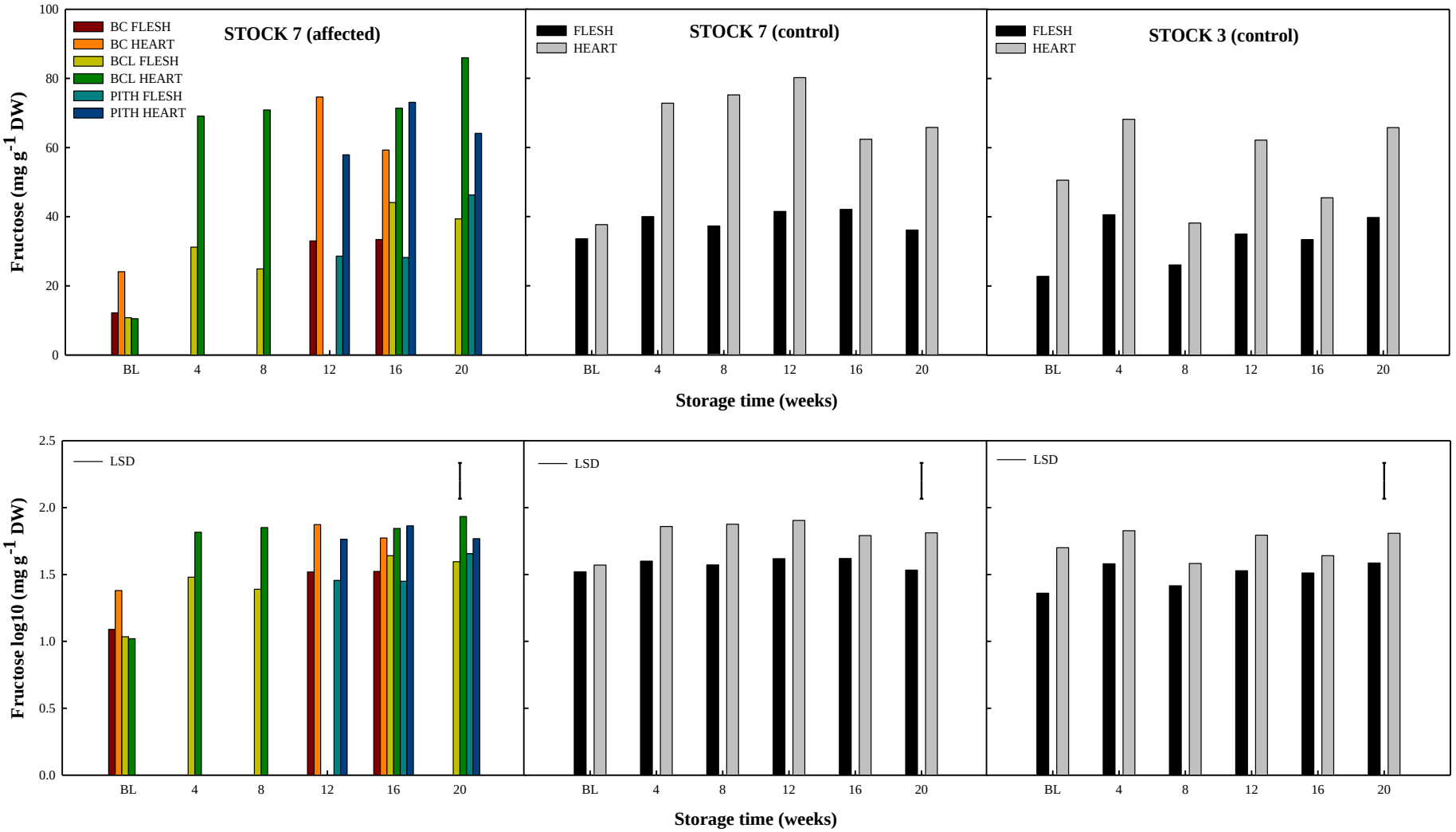

Figure 11 Fructose unlogged and logged10 concentrations (mg g^{-1} DW) in flesh and heart tissue of potato tubers Maris Piper stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) at baseline and after storage in air at 20ºC. Tubers were initially stored at 1.5ºC for 4, 8, 12, 16 and 20 weeks. Values are means (n = 3). General LSD is shown.

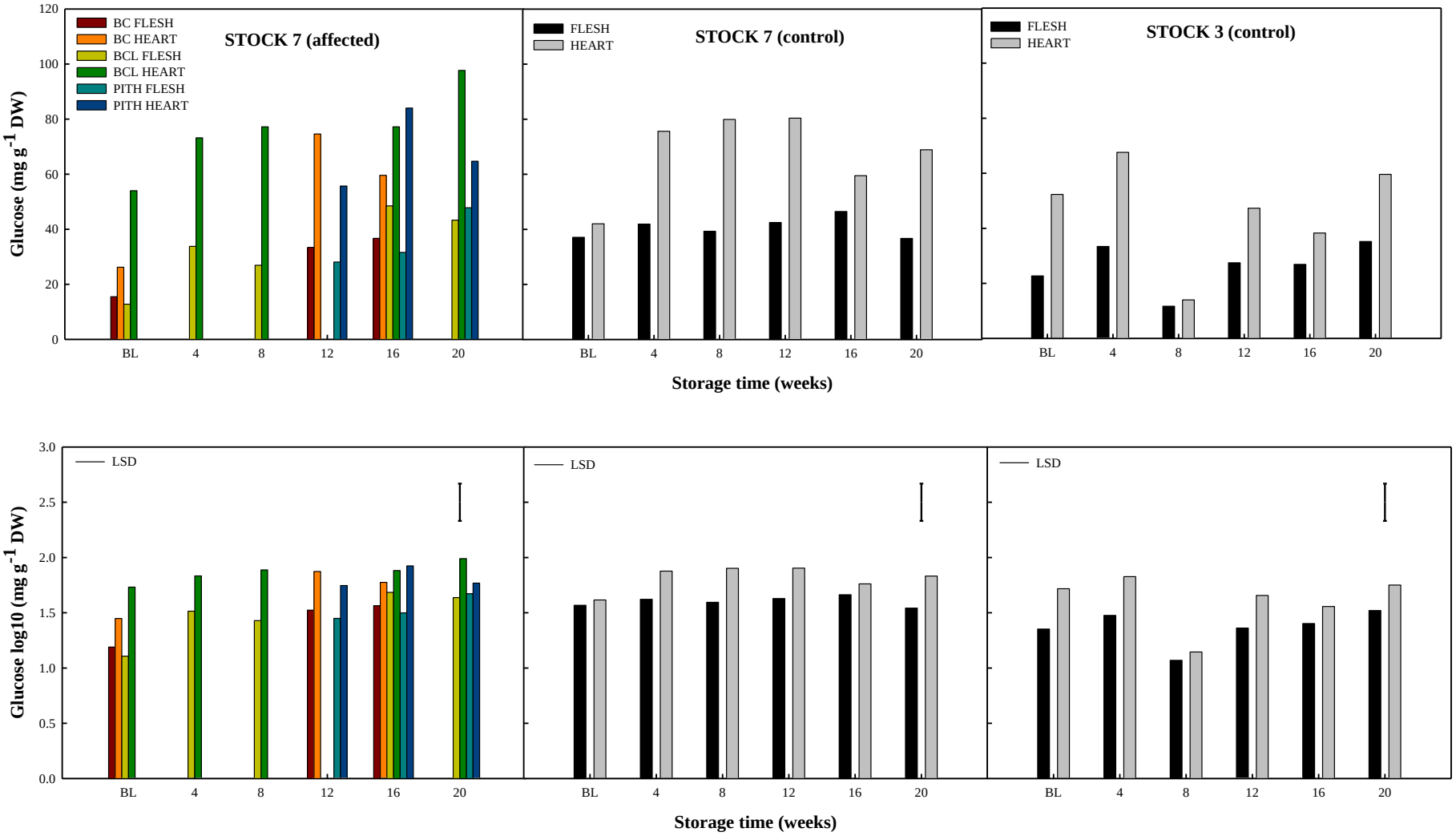

Figure 11 Glucose unlogged and logged10 concentrations (mg g^{-1} DW) in flesh and heart tissue of potato tubers Maris Piper stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) after baseline and storage in air at 20ºC. Tubers were initially stored at 1.5ºC for 4, 8, 12, 16 and 20 weeks. Values are means (n = 3). General LSD is shown.

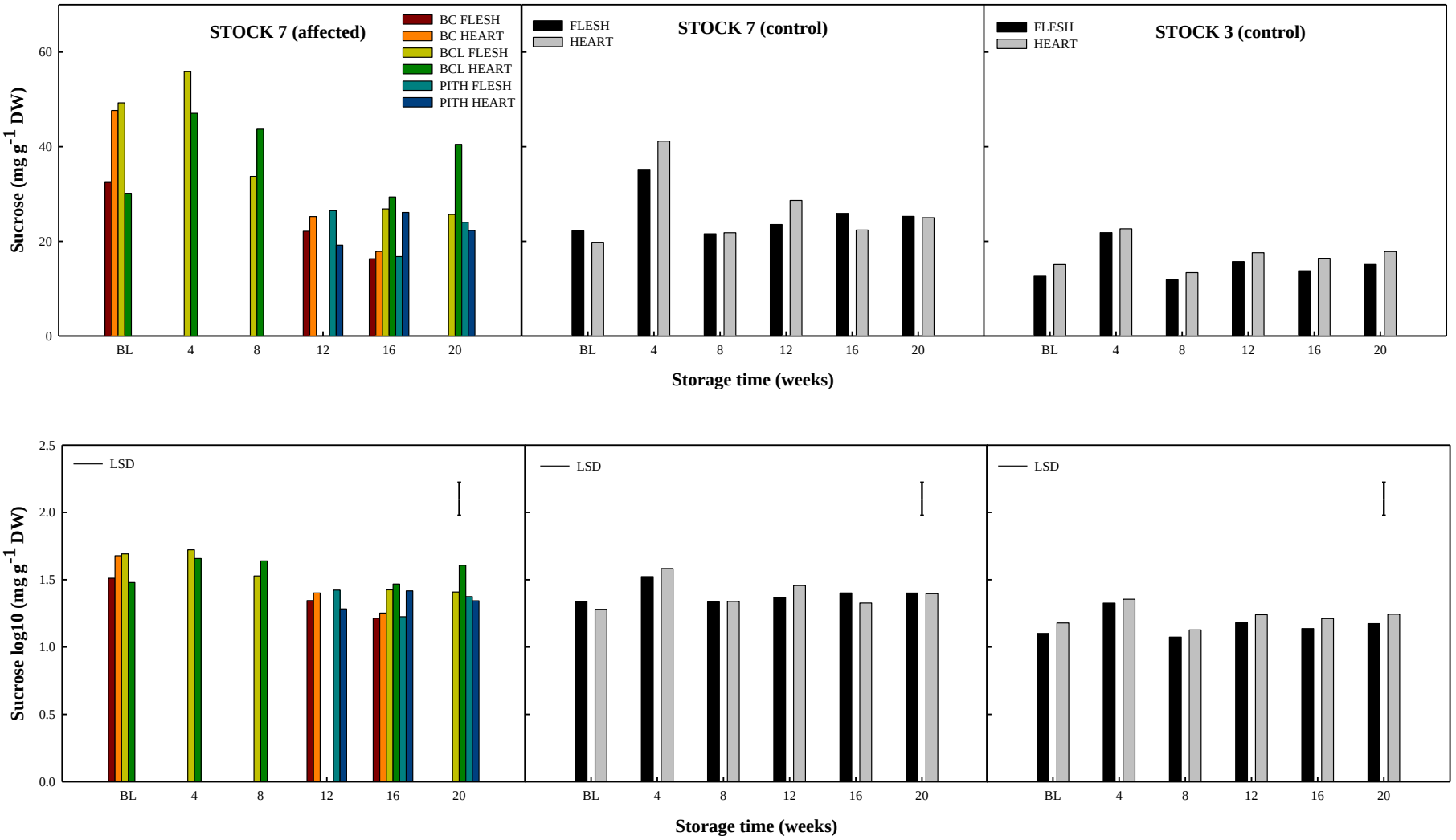

Figure 12 Sucrose unlogged and logged10 concentrations (mg g^{-1} DW) in flesh and heart tissue of potato tubers Maris Piper stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) after baseline and storage in air at 20°C. Tubers were initially stored at 1.5°C for 4, 8, 12, 16 and 20 weeks.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

To date, no relation in reducing sugar accumulation and BH disorder has ever been reported. 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 sugar content variation between the cultivars after storage at 7ºC for 2-4 months and/or after reconditioning at 20ºC for 3 weeks. The cultivars 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 1.7ºC. 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 to be susceptible to low temperature injury and BH (Craft *et al.*, 1958; Butchbaker *et al.*, 1967). Lipton (1967) reported BH incidence in White Rose potato tubers after storage in 0.5-1% O_2 at 15-20ºC 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 1ºC, but strong inhibition after storage in 1.5% O_2 at 1ºC due to hypoxia. 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.

2.5. Biochemical changes: phenolic content

Years 1 & 2

Notes on the methods

In year 1, phenolic content quantification was carried out in flesh and heart samples according to potato susceptibility to BH. Both control and discoloured samples were merged and averaged in the case of both BH susceptible stocks (20 and 23). In contrast, in year 2 phenolics were quantified in control and discoloured tissue samples separately.

Results

Chlorogenic acid was the major hydroxycinnamic acid accumulated in potato tubers. The content of chlorogenic acid varied in stocks on different sampling days.

In Year 1, flesh chlorogenic acid was ca. 2-3 times higher than in heart tissue samples of stock 20 (susceptible to BH) and stock 12 (non-susceptible to BH) while its concentration in flesh and heart of stock 23 (susceptible to BH) was about the same (Fig. 13a). 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. 13b). Crypto-chlorogenic acid was highly accumulated in flesh tissue of stock 12 (non-susceptible) compared to both susceptible stocks (stocks 20 and 23) over storage time. 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 (Fig. 13c).

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 had very low 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. There was zero abundance in isorhmanetin-3-rutinoside and isorhmanetin-3-glucoside.

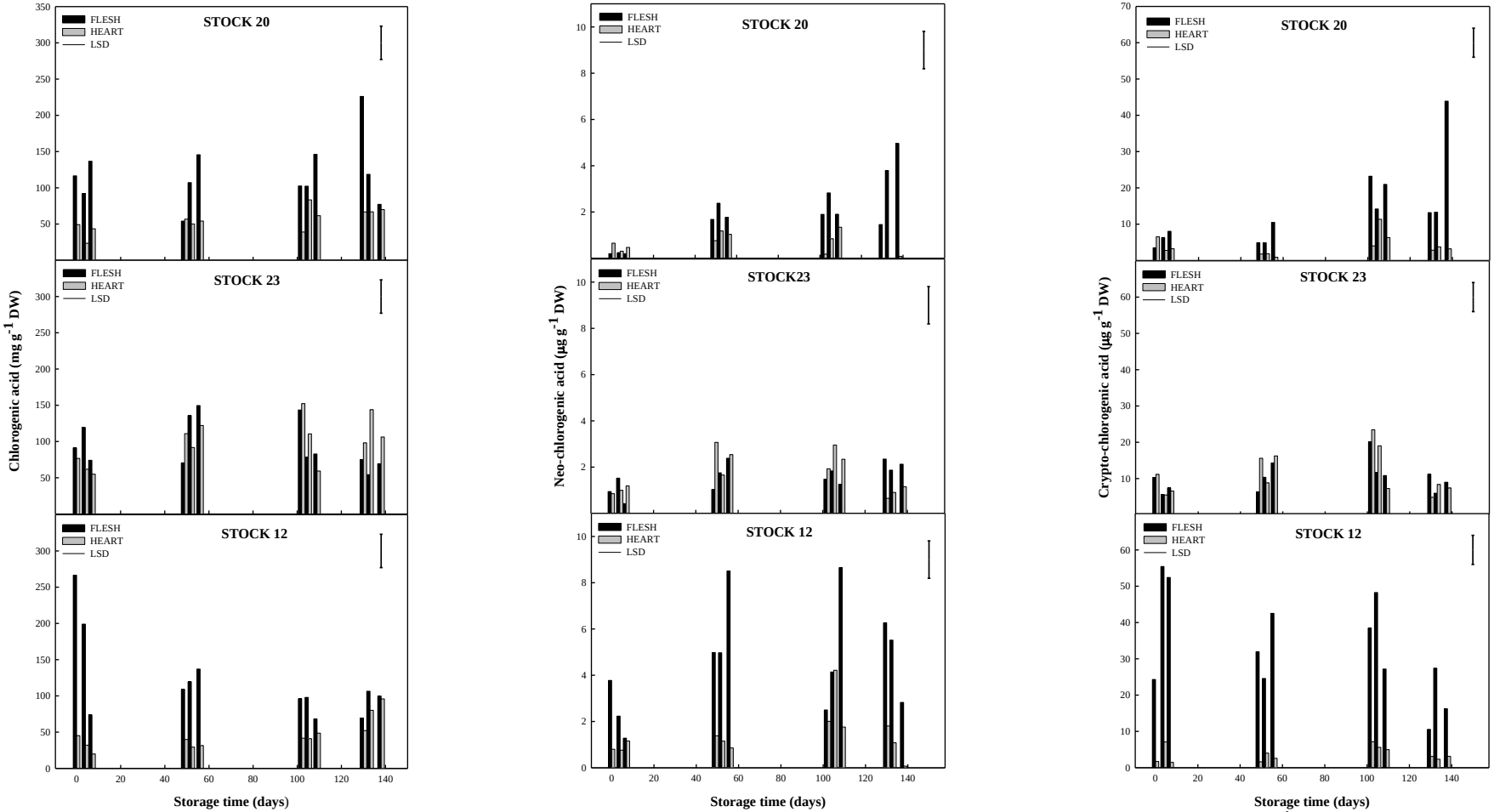

Figure 13 Chlorogenic acid (a), neo-chlorogenic acid (b) and crypto-chlorogenic acid (c) concentrations (µg g^{-1} DW) in flesh and heart tissue of potato tubers cv. Maris Piper stock 20 (susceptible to BH), stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) at baseline and after storage in air only at 15ºC on sampling days 0, 3 and 7. Tubers were initially stored at 1.5ºC for 8, 16 and 20 weeks. Values are means (n= 3). LSDs are shown ($P < 0.05$).

In year 2, the levels of chlorogenic acid recorded were higher than those in year 1. Some tubers of stock 7 (susceptible to BH) developed BH symptoms. The chlorogenic acid content was highest in the heart samples (both discoloured and control) of stock 7 (susceptible to BH) (Fig. 14). Flesh tissue samples had always higher neo-chlorogenic content compared to heart tissue over storage time and in both stocks (Fig. 15).

In this experiment, 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 experiment it was shown that chlorogenic acid tended to be more accumulated in the heart tissue samples of stocks susceptible to BH.

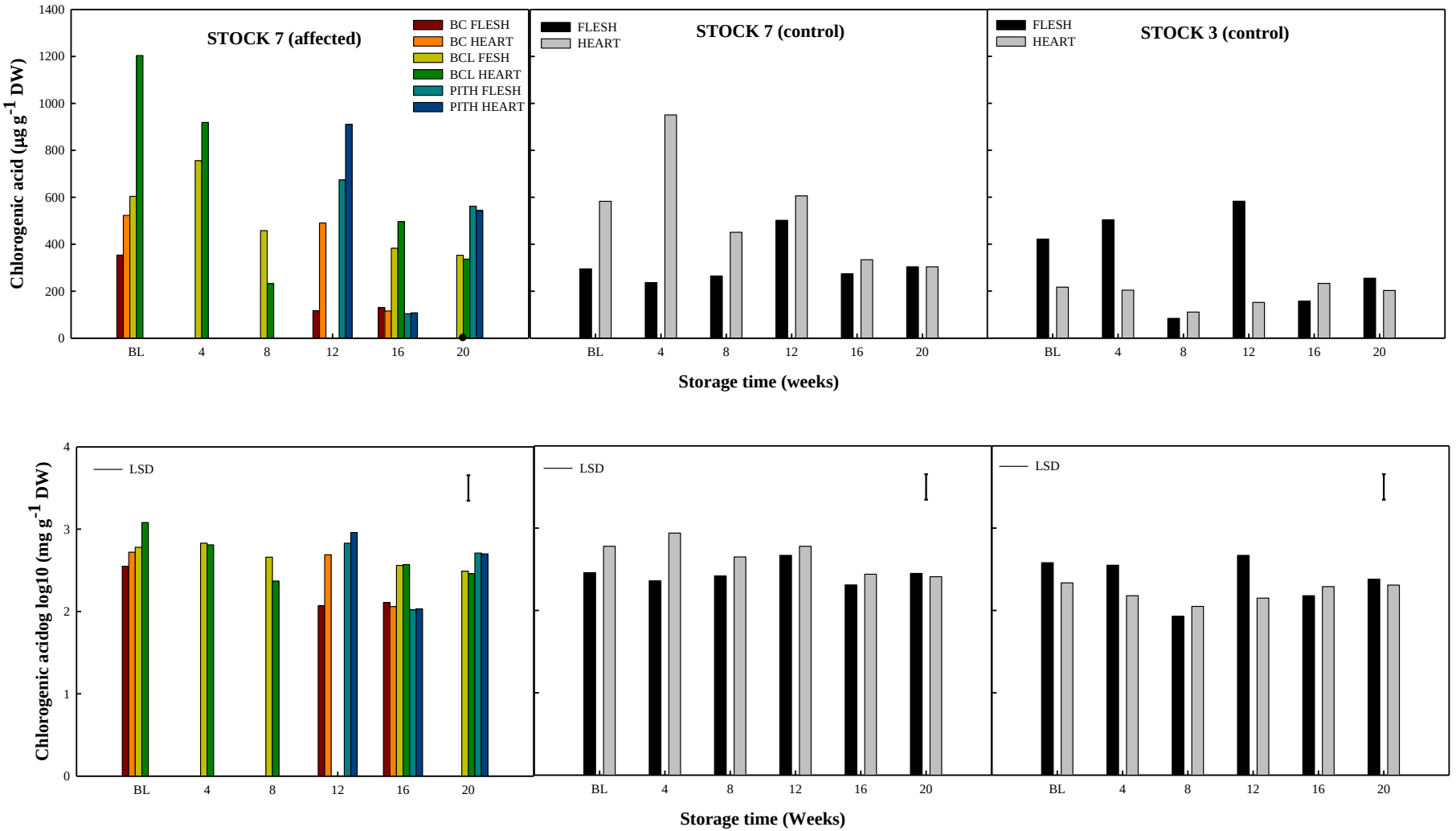

Figure 14 Chlorogenic acid unlogged and logged10 concentrations (µg g^{-1} DW) in flesh and heart tissue of potato tubers cv. Maris Piper stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) after baseline and storage in air at 20°C. Tubers were initially stored at 1.5°C for 4, 8, 12, 16 and 20 weeks. General LSD is shown.

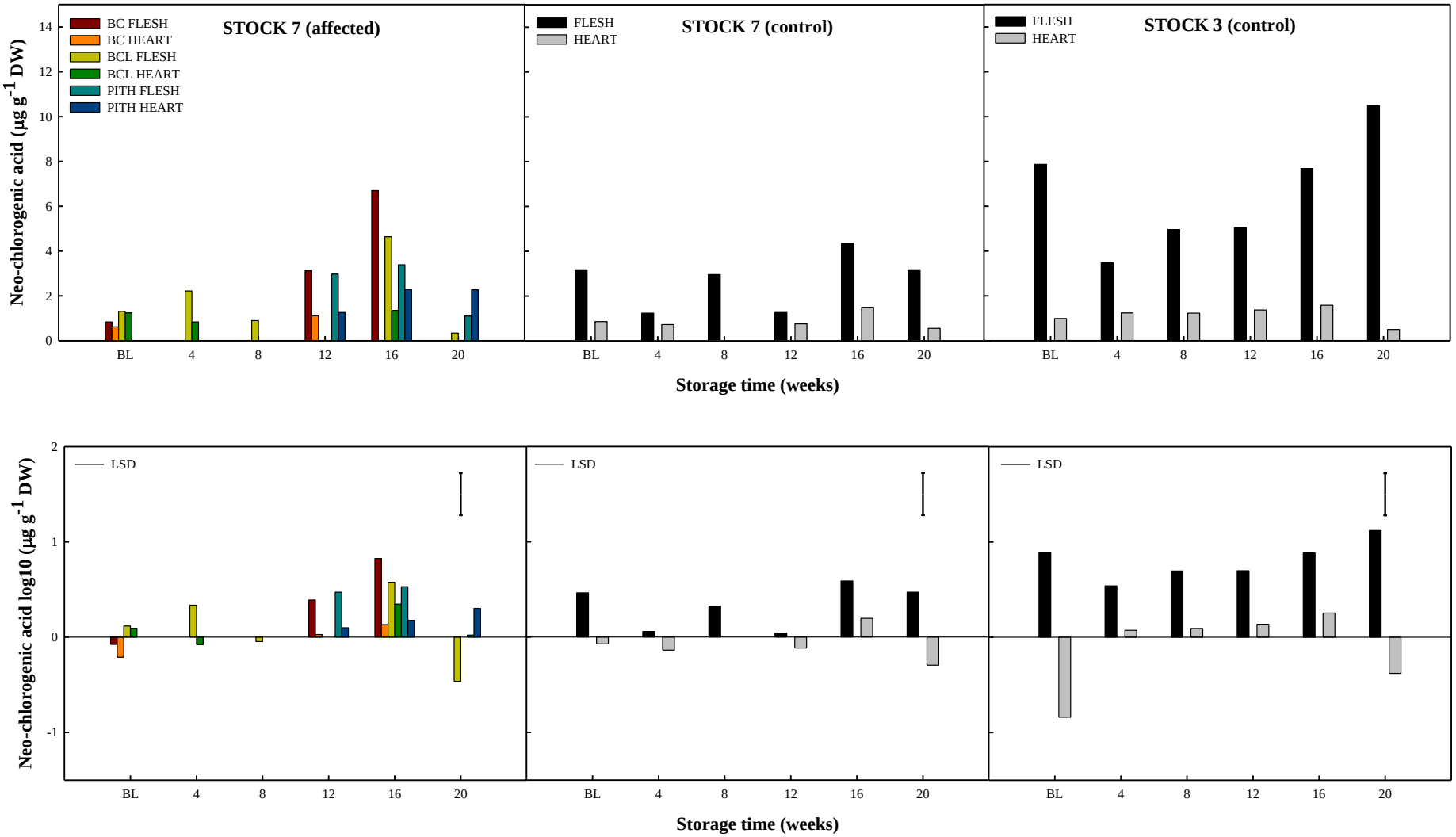

Figure 15 Neo-chlorogenic acid unlogged and logged10 concentrations (µg g^{-1} DW) in flesh and heart tissue of potato tubers cv. Maris Piper stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) after baseline and storage in air at 20ºC. Tubers were initially stored at 1.5ºC for 4, 8, 12, 16 and 20 weeks. General LSD is shown.

2.6. Biochemical changes: aromatic amino acids

In year 1, the levels of the three amino acids quantified (tyrosine, phenylalanine and tryptophan) varied over storage time (Fig. 16).

In year 2, no statistical analysis was performed for tyrosine due to the high number of missing values. However, significant differences in phenylalanine and tryptophan content were shown (Fig. 17 and 18). In general, phenylalanine was greater in heart tissue samples but significantly higher only in those samples of stock 7 (susceptible to BH).

High phenylalanine content was observed in heart samples with BC and pith discoloration and also in control heart samples of stock 7 after 16 weeks of storage. However, the lowest phenylalanine concentrations were shown in flesh and heart samples with BC after 12 weeks of storage (Fig. 17). Tryptophan was only significantly higher in heart samples with BCL after 8 and 16 weeks of storage. No significant differences between control samples of stock 7 and stock 3 were observed (Fig. 18).

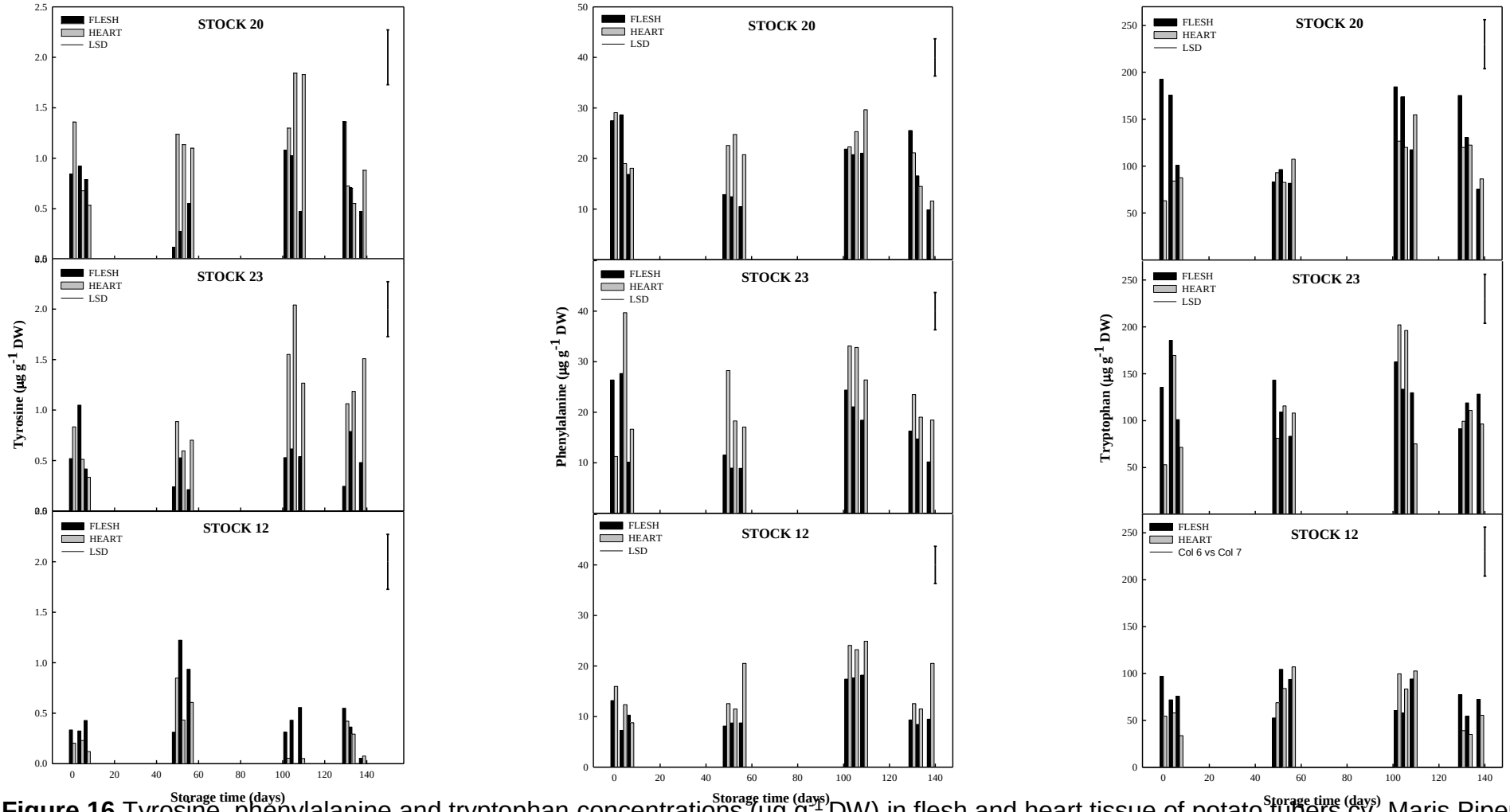

Figure 16 Tyrosine, phenylalanine and tryptophan concentrations (µg g^{-1} DW) in flesh and heart tissue of potato tubers cv. Maris Piper stock 20 (susceptible to BH), stock 23 (susceptible to BH) and stock 12 (non-susceptible to BH) at baseline and after storage in air only at 15ºC on sampling days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

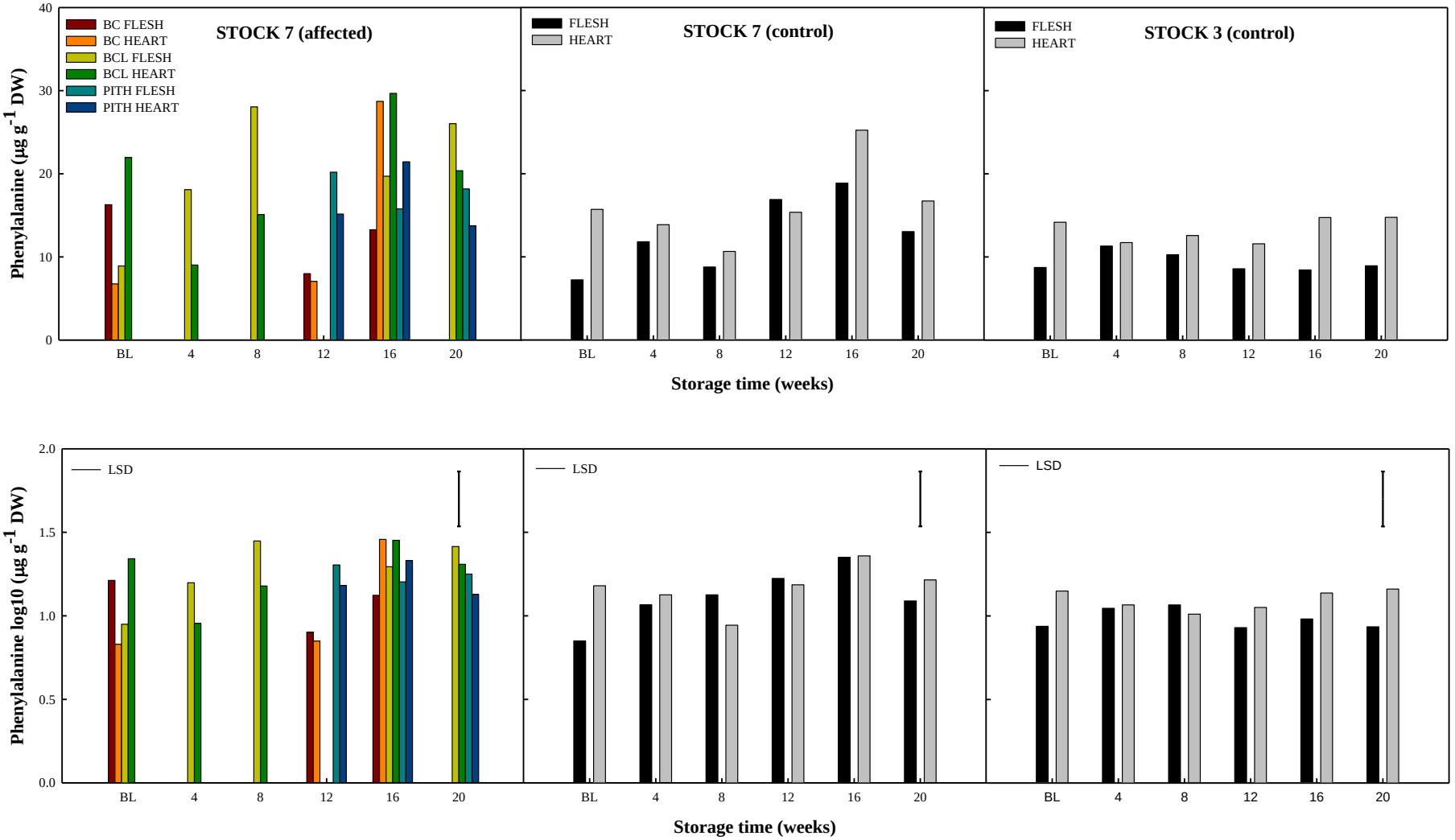

Figure 17 Phenylalanine unlogged and logged10 concentrations (µg g^{-1} DW) in flesh and heart tissue of potato tubers cv. Maris Piper stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) after baseline (0 weeks) and storage in air at 20ºC. Tubers were initially stored at 1.5ºC for 4, 8, 12, 16 and 20 weeks. General LSD is shown.

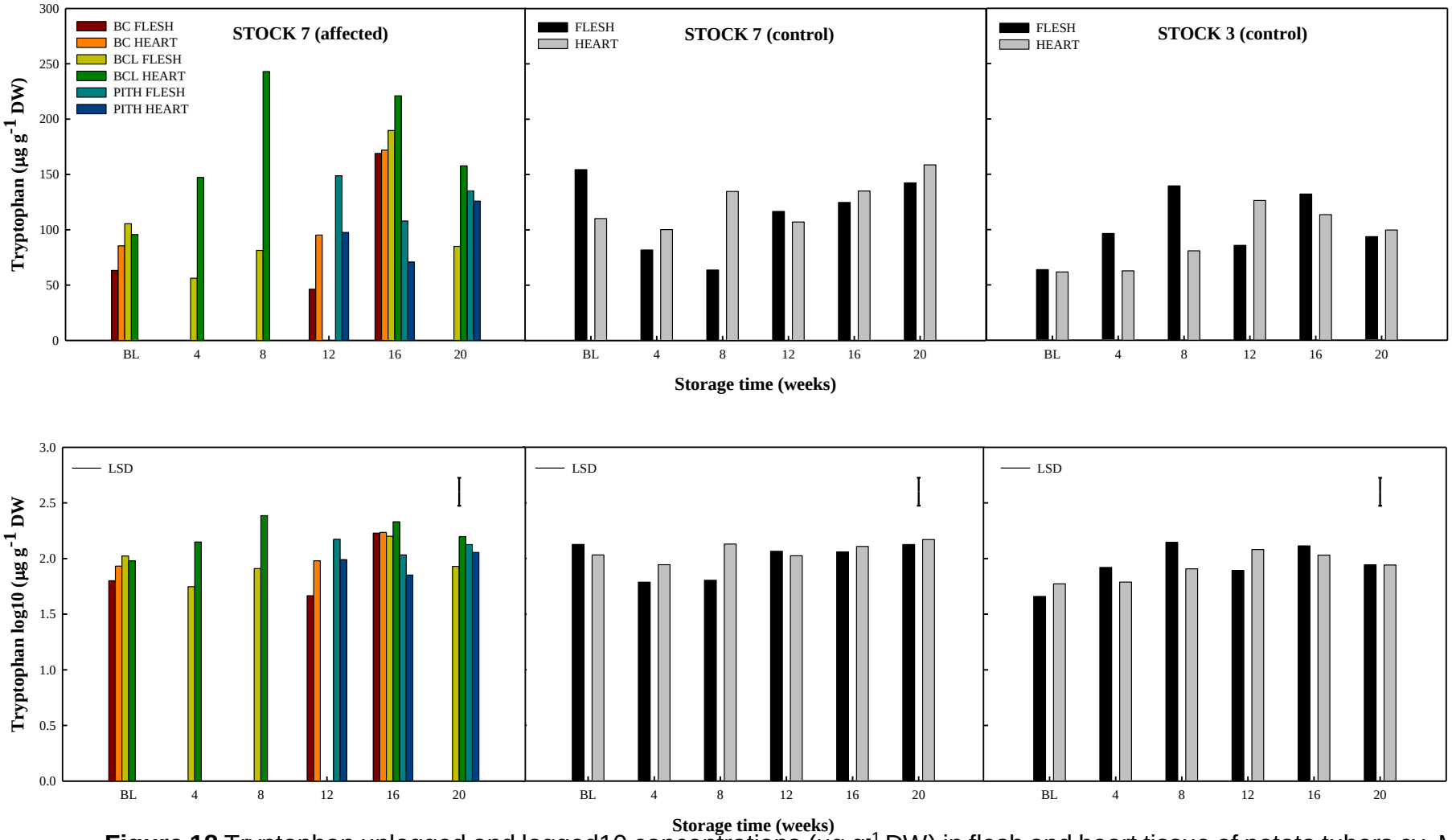

Figure 18 Tryptophan unlogged and logged10 concentrations (µg g^{-1} DW) in flesh and heart tissue of potato tubers cv. Maris Piper stock 7 (susceptible to BH) and stock 3 (non-susceptible to BH) after baseline (0 weeks) and storage in air at 20ºC. Tubers were initially stored at 1.5ºC for 4, 8, 12, 16 and 20 weeks. General LSD is shown.

Overall, a conclusion on the impact of differences in amino acid accumulation on BH incidence cannot be made. This is because there was no consistent association between levels of the different amino acids and BH in the experiments. In year 1, phenylalanine was more expressed in heart samples of stock 23 (susceptible to BH) and it was more evident after 16 and 20 weeks of storage, ie the times at which stock 23 showed greater BH incidence. Additional statistical analysis (PCA plots) indicated that tryptophan was mainly accumulated in discoloured heart samples of stock 23 after 20 weeks of storage. A similar trend in tyrosine content was followed by the heart samples of stock 23 (susceptible to BH) after 16 and 20 weeks where BH incidence was greater as well. In contrast, in year 2 phenylalanine varied in content between stock 7 (susceptible to BH) and stock 3 (non- susceptible to BH). There was also no consistent trend for tryptophan, although heart samples of stock 7 showing light brown discoloration (BCL) did tend to accumulate more tryptophan.

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.

2.7. Untargeted metabolomics approach

Untargeted metabolomics is a technique which allows the detection and identification of compounds in a sample. It provides information on which metabolites are the most highly concentrated in the sample (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 link with BH development. The purpose of this untargeted metabolomic approach was to find metabolic differences between discoloured and control samples derived from a BH susceptible 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). The descriptions were used on the assumption that less intense brown tissue discoloration may be the initial steps for the BH development (Fig. 1).

Results

Year 1

Negative ionization mode

In year 1, six samples of stock 23 (susceptible to BH) were used. In terms of the general interpretation 'discoloration only' (regardless of 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 were 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. 19). 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. 19).

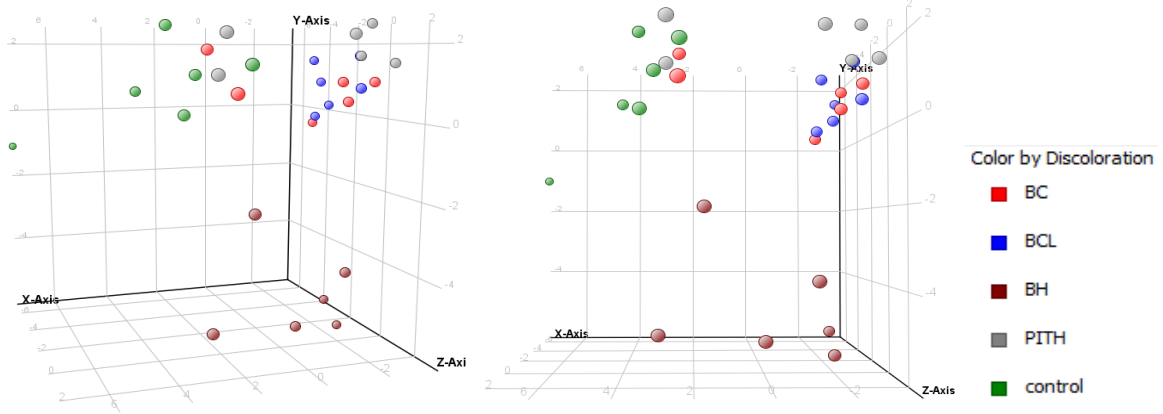

Figure 19 3D Principal Component Analysis scatter plot showing differences between tuber tissue discolorations and control of stock 23 (susceptible to BH) in negative mode (x= 27.1 %, y= 19.87%, z= 12.04%) (BC, brown centre; BCL, brown centre light; BH, dark brown to black discoloration).

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). 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. According to the figure below, it was

shown that 10 metabolites including two unsaturated hydroxyl-fatty acids, purine and pyrimidine related metabolites were all down regulated in discolorations when compared with the control (Fig. 20).

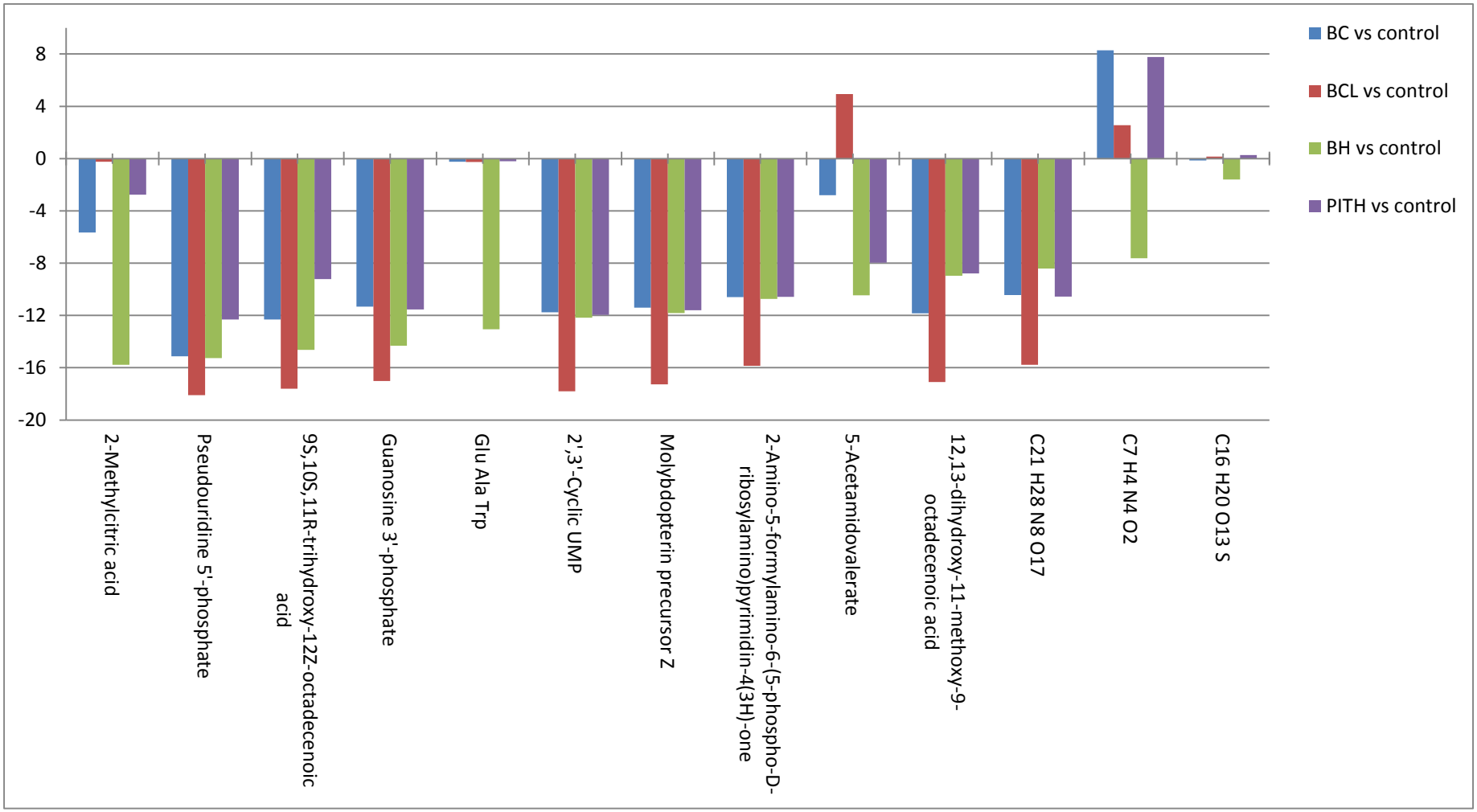

Figure 20 Fold change analysis results of 'discoloration only' interpretation in 'experiment A'. Metabolite regulation (log FC normalized) is shown in negative mode (BC, brown centre; BCL, brown centre light; BH, dark brown to black).

Positive ionisation mode

In year 1, 2516 known and un-known metabolites in total were detected. Comparison of all discolorations (BC, BCL, BH or pith) with control tissue and filtering by frequency reduced the number of total metabolites to 517. These 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) (Fig. 21). The separation of the tissue discoloration followed a similar pattern as it has also been seen in negative mode.

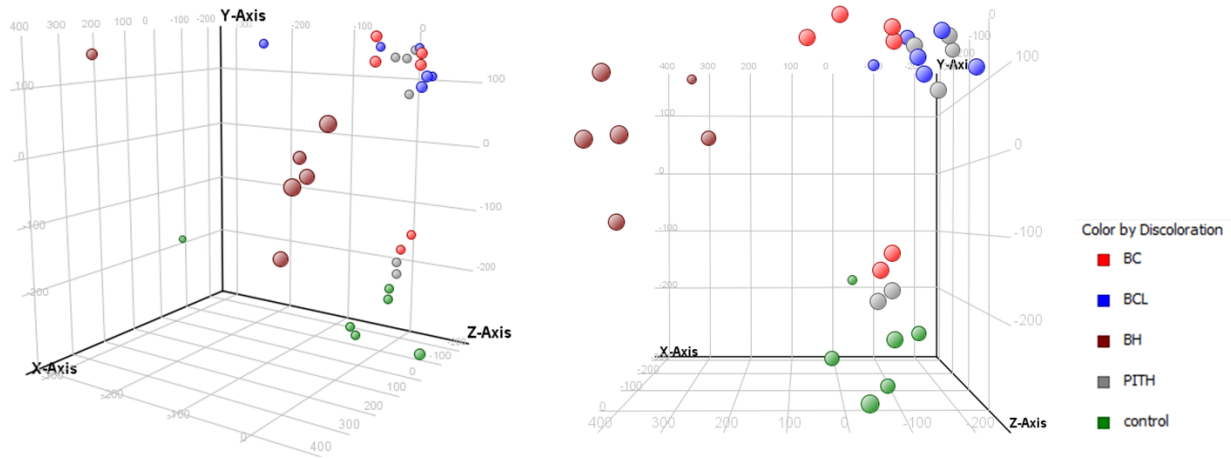

Figure 21 3D Principal Component Analysis scatter plot showing differences between control and tissue discolorations of stock 23 (susceptible to BH) in positive mode (x= 23.25%, y= 19.37%, z= 10.65) (BC, brown centre; BCL, brown centre light; BH, dark brown to black).

Fold change analysis indicated that 33 metabolites varied in regulation between the samples analysed. 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 ($P < 0.001$). Fold change analysis results showed that those 26 metabolites varied in regulation. 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 (Table 2).

Table 2 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).

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

Year 2

In year 2, 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 of 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

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.

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 (Fig. 22). However, a clear separation between affected and control samples was not distinguished.

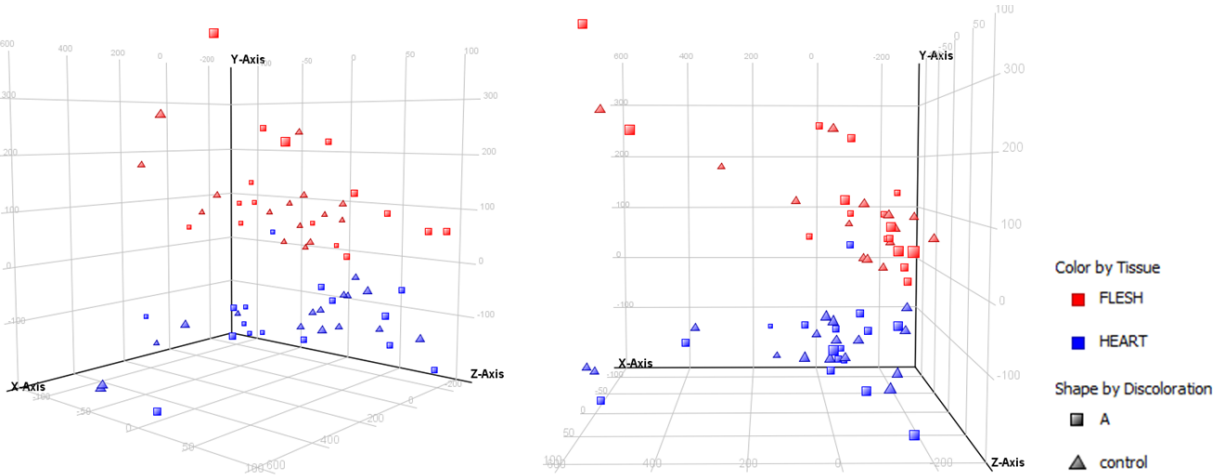

Figure 22 3D Principal Component Analysis scatter plot showing differences between affected and control tissue samples of stock 7 (susceptible to BH) in negative mode (A= affected, control = no discoloration) (x= 22.66%, y= 13.34%, z= 5.98%).

Positive ionization mode

2071 known and un-known metabolites were identified in positive mode. Interpreting the 'discoloration' condition (regardless of 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 23).

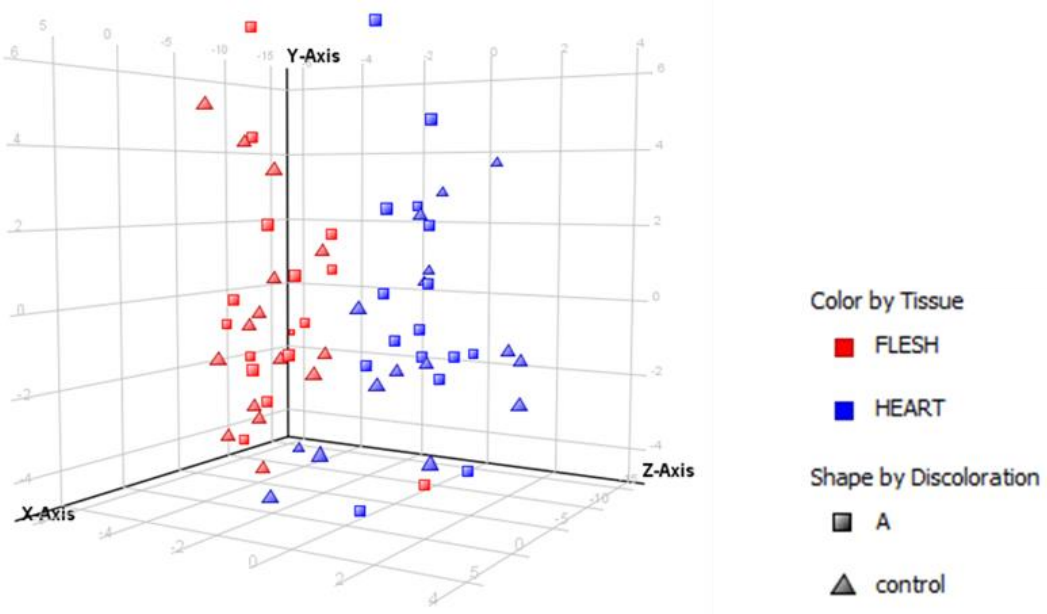

Figure 23 3D Principal Component Analysis scatter plot showing differences between affected and control tissue samples of stock 7 (susceptible to BH) in positive mode (A = affected, control = no discoloration) (x= 45.07%, y= 13.12%, z= 7.65%).

Overall, 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% was observed in both ion modes in year 1. 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.

Evidence of cell membrane lipid peroxidation and tissue damage was observed as unsaturated hydroxyl-fatty acids and lipid peroxidation products were identified. In addition, the fact that some of the unsaturated hydroxyl-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 seen. 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). 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)].

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 of 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. Glycoalkaloids are localized and accumulated in the vacuoles and the cytoplasm and may be transferred if the tissue is damaged (Väänänen, 2007). In this experiment (year 1), the results referred

to are those where unaffected tissues of stocks considered BH susceptible were compared with unaffected tissue from stocks considered susceptible to BH. No indications of glycoalkaloid accumulation in discoloured samples were observed. In year 2, 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)].

Flavonoids were identified in tissue samples in both years of this work. 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, 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 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.

3. CONCLUSIONS

The work described in this report was a component of a larger project investigating blackheart. The project also included trials at SBCSR. Those trials lead to the development of a method to induce blackheart which can be used on a commercial scale to assess the likelihood that stocks will develop blackheart symptoms. The method was also used to select stocks for more detailed biochemical and physiological studies (as described in this report). However, this proved problematic. Although the induction method will be useful in a commercial context, not all tubers within a stock designated as blackheart susceptible will ultimately develop symptoms of the condition. This has imposed limitations on the conclusions that can be drawn from this work. This is because the number of tubers (from a stock) which can be studied using the detailed biochemical and physiological techniques is limited and as such often meant that in the absence of a method to reliably induce blackheart in 100% of tubers of a susceptible stock, the researchers were forced to work with tubers that never developed blackheart symptoms. As such it has been difficult to draw firm conclusions about the factors which

contribute to or are characteristic of blackheart symptom expression. Based on the information that is available the conclusions are:

- Cold initial temperature (1.5^{o}C) was the main factor influencing both respiration rate and compositional changes in potato tubers from stocks with different susceptibility to BH.
- A relation between respiration rate and BH could not be made.
- The temperature and exposure period in which BH shows greater incidence could not readily be predicted. It is still unclear whether brown tissue discolorations seen 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.
- Similarities in biochemical changes between susceptible and non-susceptible BH stocks were observed in both years 1 and 2.
- Sugars tended to be more accumulated in the BH susceptible stocks compared to the non-susceptible stocks.
- Chlorogenic acid was highly accumulated in both control and discoloured samples of susceptible to BH stocks.
- Untargeted metabolomics lead to the identification of differences in chemical composition between samples of tissues with blackheart symptoms and those without (and between tubers from stocks designated as blackheart susceptible and those designated as non-susceptible). The differences were more pronounced in year 1 of the work. A larger number of samples were analysed in year 2 of the study and further biochemical research is needed in order to understand how differences in the composition/quantity of metabolites between tissue samples are related to blackheart susceptibility.

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5. RELEVANT LITERATURE

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