

Susceptibility to blackheart disorder in potato tubers is influenced by sugar and phenolic profile

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ABSTRACT

Blackheart (BH) is a physiological disorder of potato tubers in which internal tissue becomes discoloured during storage. The development of BH has been previously linked with general phenolic accumulation. In this study, five potato stocks cv. Maris Piper with different susceptibility to BH were selected across two consecutive seasons, whereupon targeted analysis of sugar and individual phenolic compounds in two tuber sections (flesh and heart) was conducted after storage at 1.5 °C or after one week at 15 °C. The most susceptible stock to BH had the highest accumulation of reducing sugars, while crypto- and neo-chlorogenic acids (chlorogenic acid isomers) were more abundant in flesh tissue of non-susceptible stocks. It is postulated that these metabolites may represent putative pre-symptomatic predictive biomarkers of stock susceptibility to BH.

1. Introduction

Potato blackheart (BH) is a postharvest disorder, which was initially reported by Bartholomew (1916) in shipped potatoes, and still results in significant losses for the potato industry (Kiaitsi, 2015). BH-affected tubers remain firm and odourless, without external symptoms, and the disorder is only observed when tubers are sliced open. Thus, tubers may pass quality control checks and be marketed, with defects only becoming apparent to the consumer during preparation or prior to consumption. Customer complaints associated to internal discoloration in potato are significant and have risen over the last decade in the UK (Kiaitsi, 2015; Terry, 2015). The disorder has been associated with O₂ depletion and/or CO₂ accumulation in internal tissue (Hooker, 1981) but it has not been clearly characterised. BH is a non-pathogenic disorder appearing as internal brown-to-black discoloration; it mainly affects medullary tuber tissues without reaching the cortex and may result in cavity formation (Hooker, 1981). Due to variable degrees of tissue discoloration (brown or black, severity-related) the disorder can be mistakenly referred to as incipient brown centre (BC), brown heart, hollow heart (HH) or sugar heart (SH) (Bussan, 2007). This diversity in nomenclature has led to confusion over the identification of the factors which relate to the specific disorder (Reeve, 1968; Sowokinos, 2007). Disorders such as BC, HH might coexist with BH or act as its precursor (Hooker, 1981).

Near freezing or high (> 32 °C) temperatures have been reported to play a role in BH development during storage, likely as a result of

different gas diffusion properties within tuber tissues (Stewart and Mix, 1917). It has been reported that BH development requires longer storage times at colder temperatures (0–2.5 °C) (Link and Ramsey, 1932). More recently, Zhou et al. (2015) reported that BH can be induced in tubers enclosed in zip-lock bags (carbon dioxide accumulation and oxygen depletion) when stored at 4 °C for four months.

The brown to black coloration associated with BH indicates a phenolic-based reaction, and indeed Bartholomew (1916) suggested that tyrosine oxidation, via polyphenol oxidase (PPO), might be responsible for the tissue discoloration. Reeve (1968) demonstrated that suberin and other phenolics accumulated in the parenchyma of BH affected tubers compared to symptomless tubers. It is possible that, together with PPO, phenylalanine ammonia lyase (PAL) plays a role in the brown pigmentation development, since PAL activity is affected by gaseous stress (Joos and Hahlbrock, 1992; Geigenberger, 2003). Nevertheless, no direct correlation between potato phenolics and BH susceptibility has been described. In fact, further research has mainly focused on non-destructive detection methods of the disorder rather than biochemical profiling during development (Zhou et al., 2015; Mohamed et al., 2016; Tian et al., 2017).

In the current work, targeted metabolomic approaches were applied to investigate putative predictive biomarkers of BH susceptibility in potato tubers allowing improved management of crops for possible reduction in customer complaints.

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2. Materials and methods

2.1. Plant material and experimental design

During two consecutive growing seasons, a total of five stocks of potato (*Solanum tuberosum* L.) cv. Maris Piper (supplied by Fresh Potato Suppliers Association, FPSA, UK) with different susceptibility to BH were analysed [season 1 (2011–2012), stocks A, B, and C; season 2 (2012–2013), stocks E, and D]. Tuber size ranges between 85–110 mm in length, 68–78 mm in diameter, and 198–270 g in weight. To establish the BH susceptibility of each stock, tubers underwent a “hot-box” procedure developed and carried out at Sutton Bridge Crop Storage Research (SBCSR, UK): unwashed tubers, were initially stored at 3.5 °C and incubated in sealed chambers at 30 °C for 60 h. After incubation, tubers were longitudinally cut in half, placed on trays at room temperature for 24 h, and inspected by SBCSR staff for symptoms of BH (tissue discoloration). Following the hot-box treatment, stocks A, B and D were classified as susceptible, while C and E did not show any discoloration and were ranked as non-susceptible. Tubers of each stock were collected from SBCSR and transported to Cranfield University (CU) across the two consecutive seasons. During each season, tubers were assessed at four time points [0 (baseline, arrival at CU), 8, 16 and 20 weeks] after cold storage (1.5 °C) or after one week under shelf-life conditions (15 °C). At each time point, tubers (9 tubers/stock) were removed from cold storage and assessed as day zero (d0), while another set of tubers (18 and 9 tubers/stock in season 1 and in season 2, respectively) were transferred to 15 °C in air for one week and assessed after seven days (d7) for shelf-life evaluation. Baseline measurements were conducted on tubers of each stock before cold storage (1.5 °C). A total of 108 and 72 tubers per stock were used in season 1 and season 2, respectively. Shelf-life evaluation was conducted in 300 L boxes flushed with air (O₂; 21 %, CO₂; 0.04 %) controlled by the ICA6000 system (International Controlled Atmosphere Ltd., Paddock Wood, Kent, UK) (Amoah et al., 2017).

2.2. Stock susceptibility evaluation

On each sampling day (d0 and d7), tubers were carefully washed and left to air dry. One slice (10 mm in thickness) was longitudinally cut from the central part of each tuber with a sharp knife. Tubers slices were visually evaluated for tissue discoloration (BH-like symptoms; Fig. 1). Tissue discoloration evaluation was used to confirm or reject the previous rank following the hot-box procedure, and to evaluate the development of BH. The slices were then peeled and used for further analysis.

After discoloration evaluation, two sets of samples were individually cut from each slice: an inner section (heart) of 24 mm in

diameter, using a cork borer, including central medullary (pith) tissues; and an outer section (flesh), consisting of pith, perimedullary and cortex tissues (Fig. 2). The peel was discarded, and samples were immediately snap frozen in liquid nitrogen and freeze-dried in the dark at –50 °C using a digital freeze drier (Scanvac, Lyngø, Denmark) for seven days.

2.3. Targeted biochemical analysis

The freeze-dried samples were used for sugars and individual phenolic quantification in both sets of tissue samples (flesh and heart). During season 1, the samples were collected on every sampling day (d0 and d7) at each time point after the visual inspection (stocks A, B, and C; total of 648 samples), and evaluated following two different approaches: *i*) an analysis of biochemical variations related with BH-susceptibility, and *ii*) a snapshot of BH incidence-related profiles. Samples collected during season 2 were used to integrate the dataset for the incidence-related analysis. The tubers were individually inspected and ranked at every time point, while the tissues were sampled (from all the tubers at that time points) when a discoloration was present (stocks E and D; total of 144 samples).

To identify possible biomarkers of BH-susceptibility (*i*), the biochemical profiles of tuber tissues were investigated during storage. Due to the relatively low incidence of BH, the tubers sampled at each sampling point were individually ranked for discoloration, and the freeze-dried tissues were treated as replicates/stock (3 tubers each rep, 3 reps per stock), regardless of BH incidence. As a result, discolored and non-discolored tissues of susceptible stocks (A, B and D) were merged when presented at the same time point. The identified differences were therefore related with the stock susceptibility rather than discoloration per se.

To study possible biomarkers related to BH-incidence (*ii*), samples of each susceptible stock of both seasons were grouped into discolored vs. non-discolored (regardless of the time point), compared between them, and against the non-susceptible tuber tissues (stock C and E).

2.3.1. Non-structural carbohydrate extraction and quantification

Non-structural carbohydrates (fructose, glucose and sucrose) were extracted and analysed as described by Terry et al. (2007) using a High-Performance Liquid Chromatography Agilent 1260 series coupled to Infinity Evaporative Light Scattering Detector (ELSD) (Cheshire, UK). Diluted extracts (1/4) were injected into a Prevail Carbohydrate ES u (GRACE) 250 mm × 4.6 mm column. Sugar concentrations were calculated against authentic calibration standards of fructose, glucose and sucrose (Sigma, Dorset, UK) ranging from 0.1 to 5 g L⁻¹ and the results were expressed as g kg⁻¹ of dry weight (DW).

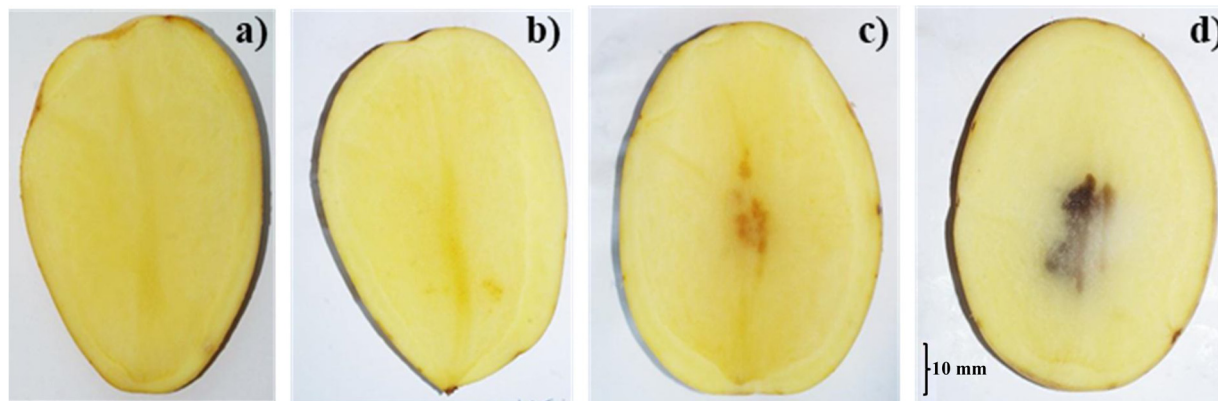


Fig. 1. Longitudinally sliced potato tubers with different intensity in tissue discolorations. a) no discoloration b) pith discoloration. c) brown discoloration. d) dark brown to black discoloration. Apical end at the top and stolon end at the bottom.

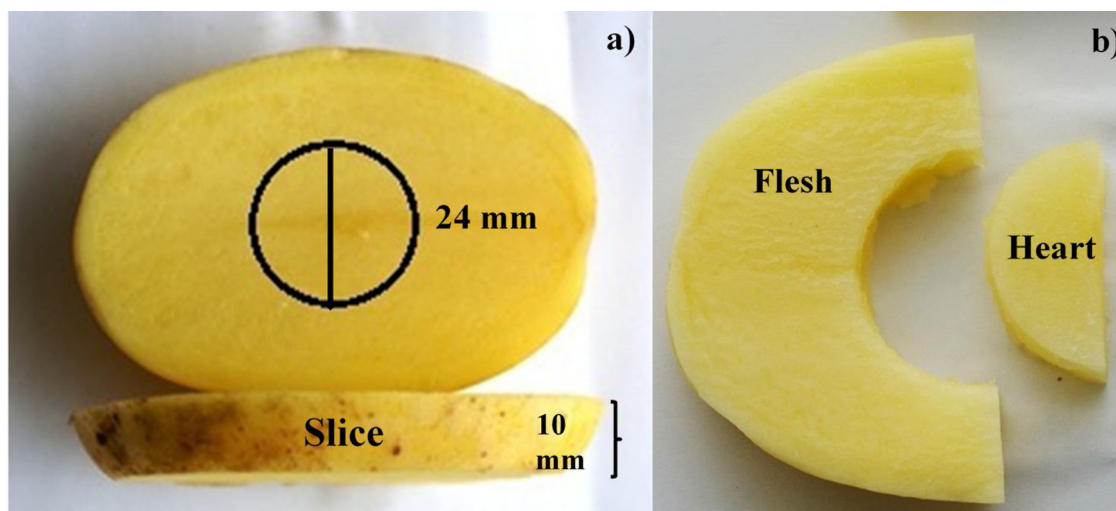


Fig. 2. Tuber slice (10 mm in thickness) (a), flesh and heart samples derived from stolon end of the tuber (24 mm in diameter) (b).

2.3.2. Phenolic extraction and quantification

Individual phenolics were extracted from 50 mg freeze-dried material. Samples were extracted with 1.5 mL of 50:50 LCMS grade methanol:water (v/v) + 1 % formic acid solution and then placed in a shaking water bath at 35 °C for 15 min and every 5 min they were removed and vortexed (Vortex Genie 2, Scientific Industries, NY) at room temperature for ca. 20 s. After incubation, samples were centrifuged for 10 min at 10,000 rpm and the supernatant collected and filtered through a 0.2 µm filter (Cronus PTFE filters, Jaytee Biosciences Ltd., Kent, UK). Individual phenolic compounds of samples (10 µL injection) were measured 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. Chromatography was performed on a WATERS – ACQUITY UPLC C18 2.1 × 150 mm 1.7 µm column (WATERS, Ireland) 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. 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 calculated in mg kg⁻¹ DW. Each external standard of [chlorogenic acid (5-O-caffeoylquinic acid), crypto-chlorogenic acid (3-O-caffeoylquinic acid), neo-chlorogenic acid (4-O-caffeoylquinic acid), tyrosine, phenylalanine, rutin (quercetin-3-O-rutinoside), and quercetin-3,4-O-diglucoside] (Sigma, Dorset, UK) was dissolved with 50:50 methanol:water (v/v).

2.4. Statistical analysis

Non-structural carbohydrates and individual phenolic data sets were analysed by general Analysis of Variance (ANOVA) performed with GenStat 16th Edition (VSN International Ltd, Herts., UK). Raw data were checked for residuals distribution; ANOVAs were followed by a comparison of the means according to the least significant difference (LSD) test ($P < 0.05$).

3. Results

3.1. Stock susceptibility

Visual assessment data on tissue discoloration confirmed the differences in degree of susceptibility among the stocks as indicated by SBCSR, and no discoloration was detected in the two non-susceptible stocks (C and E) at any time point (Table 1). Generally, greater susceptibility was observed in stocks B (season 1) and D (season 2) showing up to a 6-fold difference compared to stock A (Table 1).

Tissue discoloration incidence was first observed during shelf-life evaluation at week 0 (baseline) in stock D and peaked after 16 weeks' storage (Appendix A; Supplementary Table 1). BH incidence increased after seven days (d7) shelf-life at 15 °C.

3.2. Biomarkers of BH-susceptibility

At baseline, no significant differences in sugar contents were detected among season 1 stocks A (least susceptible), B (most susceptible) and C (non-susceptible), nor between the separate tissues analysed (heart and flesh). As expected, reducing sugars accumulated during cold storage at 1.5 °C (cold-induced sweetening) and this behaviour was more evident in the most susceptible stock (B). In particular, stock B showed a marked accumulation of glucose and fructose in both tissues throughout cold storage (Fig. 3, line and scatter plots). In addition, a similar pattern was still identified for those similar tubers assessed after one week of shelf-life at 15 °C. The tubers of stock B also exhibited significantly high reducing sugars concentrations compared to the other

Table 1

Total incidence of tissue discoloration of potato tubers cv. Maris Piper and total percentage of stock susceptibility to BH (season 1, stocks A, B, and C; season 2, stocks D, and E).

| STOCK | TOTAL TUBERS | TOTAL DISCOLORATION | % ^a | % ^b | SUSCEPTIBILITY RANK (CU) |
|-------|--------------|---------------------|----------------|----------------|--------------------------|
| A | 108 | 3 | 74.0 | 2.8 | least susceptible |
| B | 108 | 18 | 80.0 | 16.7 | most susceptible |
| C | 108 | 0 | 0.0 | 0.0 | non-susceptible |
| D | 72 | 11 | 18.8 | 15.3 | susceptible |
| E | 72 | 0 | 0.0 | 0.0 | non-susceptible |

^a Total percentage of BH symptoms incidence reported by SBCSR following the hot box method.

^b Total percentage of BH symptoms incidence reported by CU after 20 weeks of storage.

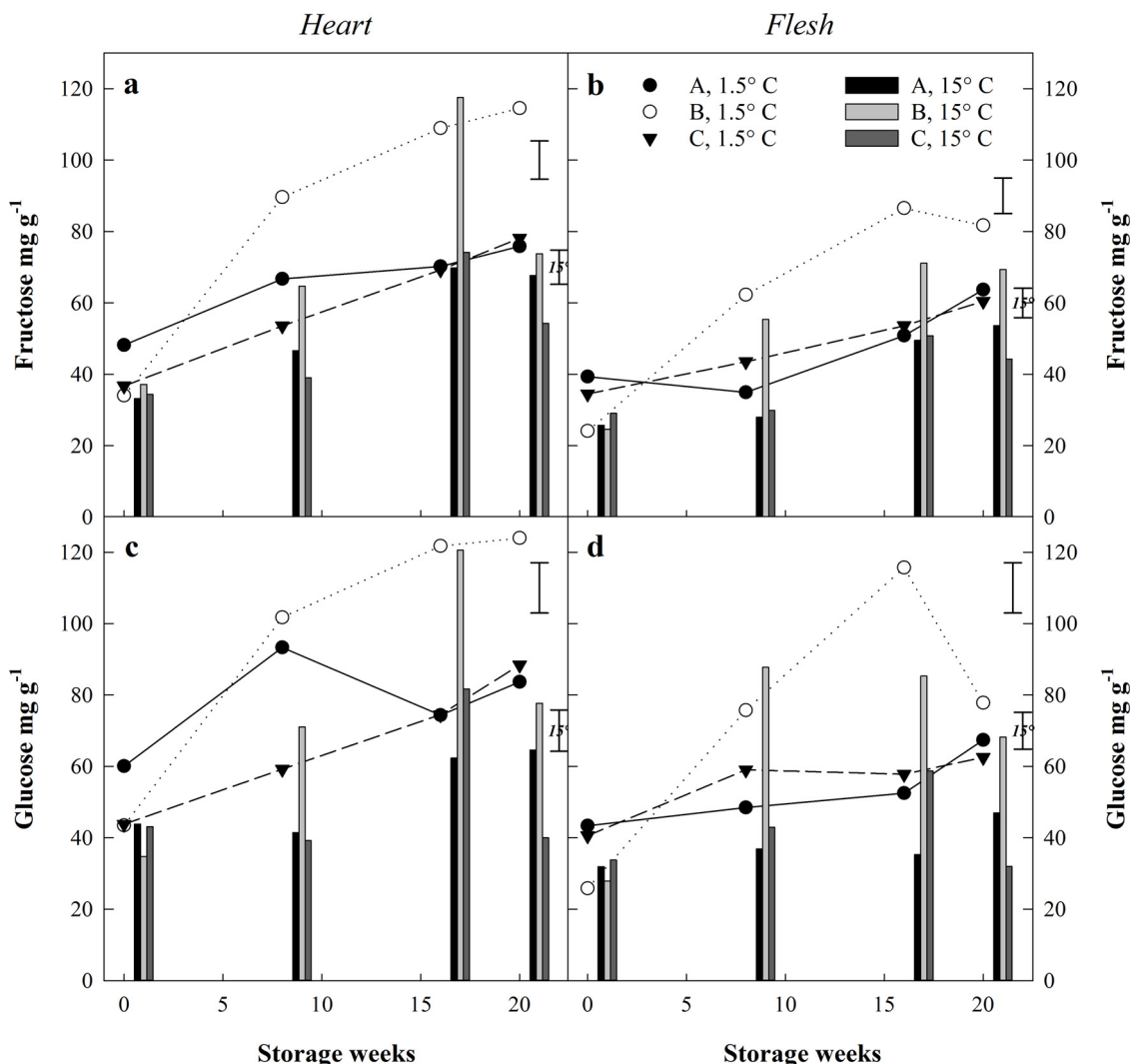


Fig. 3. Fructose and glucose (g kg^{-1} DW) in flesh and heart samples of stocks A (least susceptible), B (most susceptible), C (non-susceptible) during cold storage (1.5°C ; line and scatter plots), and after one week of shelf-life (15°C ; bar plots) (baseline; week 0). LSD bars are shown ($P < 0.05$).

stocks analysed (Fig. 3, bar plots). Sucrose contents were also investigated but no specific patterns were identified between stocks of different susceptibility.

In terms of the amino acids, tyrosine exhibited an upwards trend in the heart tissue of non-susceptible stock C during cold storage, while its concentration fluctuated in the susceptible stocks A and B (Fig. 4a, line and scatter plots). A peak in tyrosine accumulation was detected at week 16, and during shelf-life, in both susceptible stocks (Fig. 4a, bar plots). Phenylalanine accumulated in heart tissues of the most susceptible stock (B) but after shelf-life (15°C) no differences were found among stocks (Fig. 4b). Chlorogenic and crypto-chlorogenic acids followed a similar pattern of accumulation like glucose and phenylalanine in heart samples of the most susceptible stock B throughout the cold storage (Fig. 4c and d).

In contrast, the flesh tissue of the non-susceptible stock accumulated more chlorogenic acid isomers (neo- and crypto-chlorogenic acids) than the susceptible stocks, during both cold storage and shelf-life (Fig. 5).

For BH-incidence interpretation, and because of the sporadic nature of the disorder, the flesh and heart samples of all stocks were grouped into discolored and non-discolored (regardless of the time point), and the biochemical profiles compared. In particular, reducing sugars mainly accumulated in heart samples of all stocks but the highest accumulation was recorded in both discolored flesh and heart samples of

stock B which showed the greatest BH incidence (Fig. 6). Crypto- and neo-chlorogenic acids were ca. triple and double the concentration, respectively, in flesh samples of both non-susceptible stocks (C and E) when compared to heart samples (Fig. 7). In addition, rutin and quercetin-3,4-O-diglucoside were twice as high in non-susceptible stock E (Appendix A; Supplementary Fig. 1) than susceptible stock D.

4. Discussion

Blackheart development in potato tubers can occur at any temperature where there is inadequate oxygen supply. Storage at very low or high temperatures slows down gaseous diffusion in the internal tissue and it is believed that longer periods of storage are required for BH development at cold temperatures (Stewart and Mix, 1917; Link and Ramsey, 1932; Zhou et al., 2015). The results of the current study confirmed that a storage temperature as low as 1.5°C affected the tissue discoloration of susceptible stocks (A, B and D). Moreover, the results herein highlighted that shelf-life conditions at 15°C (following cold storage) played a major role in eliciting discoloration. Therefore, it is reasonable to suggest that very low storage temperatures may trigger the mechanisms responsible for discoloration, which are then exacerbated during shelf-life. An extended shelf-life period, perhaps under low oxygen, might further enhance BH development and intensify its symptoms.

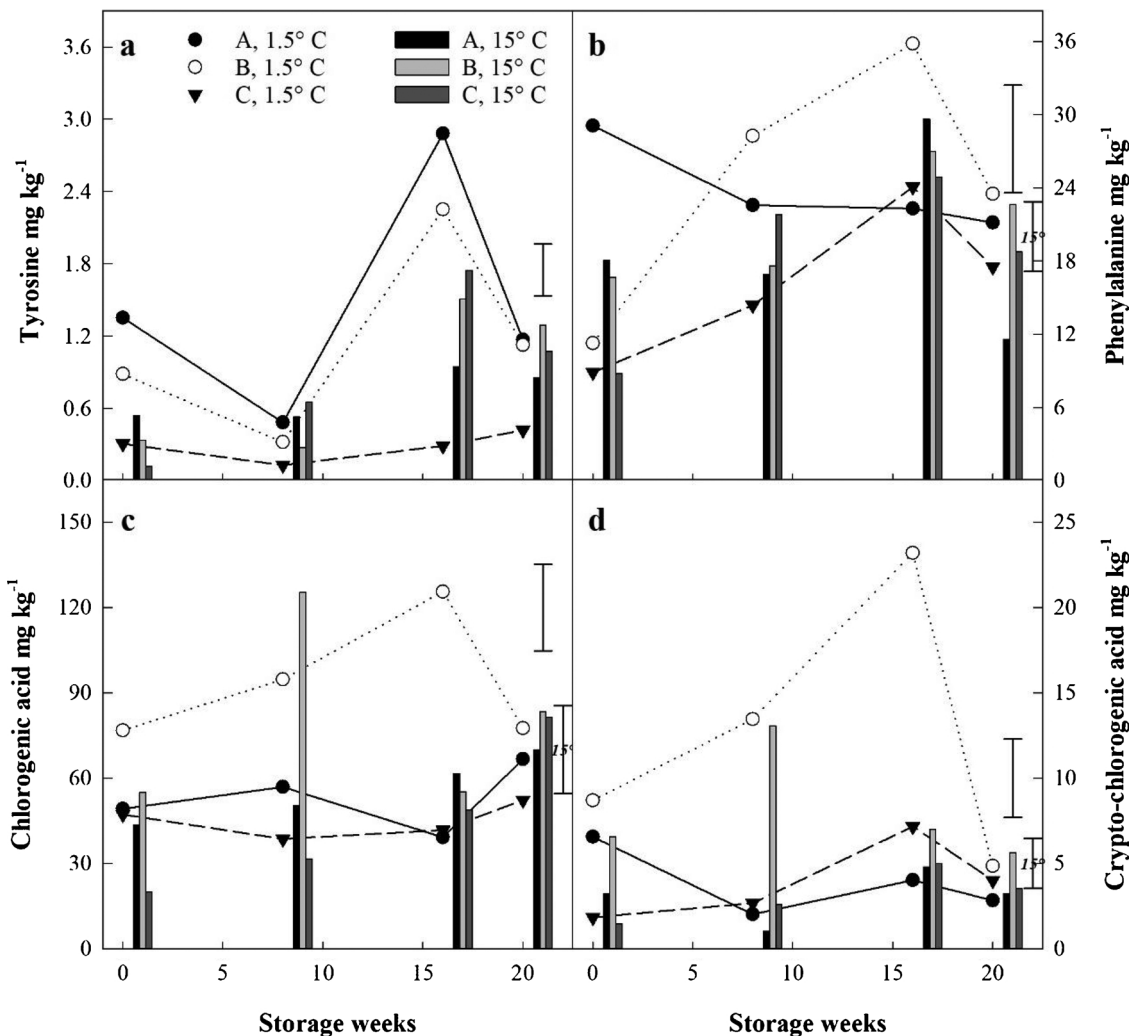


Fig. 4. Tyrosine, phenylalanine, chlorogenic acid and crypto-chlorogenic acid (mg kg^{-1} DW) in heart samples of stocks A (least susceptible), B (most susceptible) and C (non-susceptible) after cold storage (1.5°C; line and scatter plots), and after one week of shelf-life (15°C; bar plots) (baseline; week 0). LSD bars are shown ($P < 0.05$).

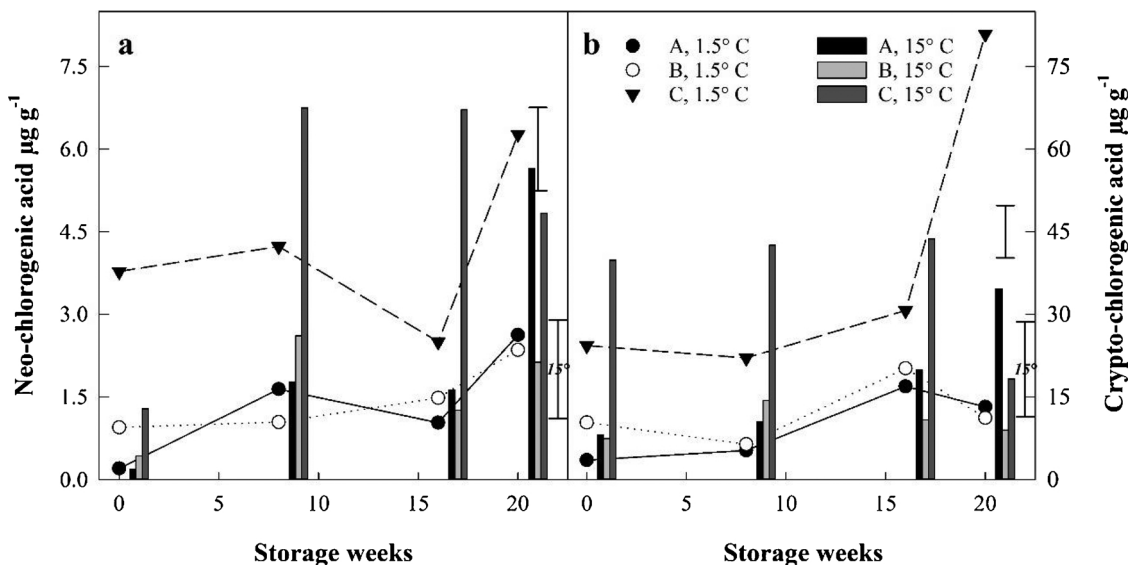


Fig. 5. Neo- and crypto-chlorogenic acids (mg kg^{-1} DW) in flesh samples of stocks A (least susceptible), B (most susceptible) and C (non-susceptible) after cold storage (1.5°C; line and scatter plots), and after one week of shelf-life (15°C; bar plots) (baseline; week 0). LSD bars are shown ($P < 0.05$).

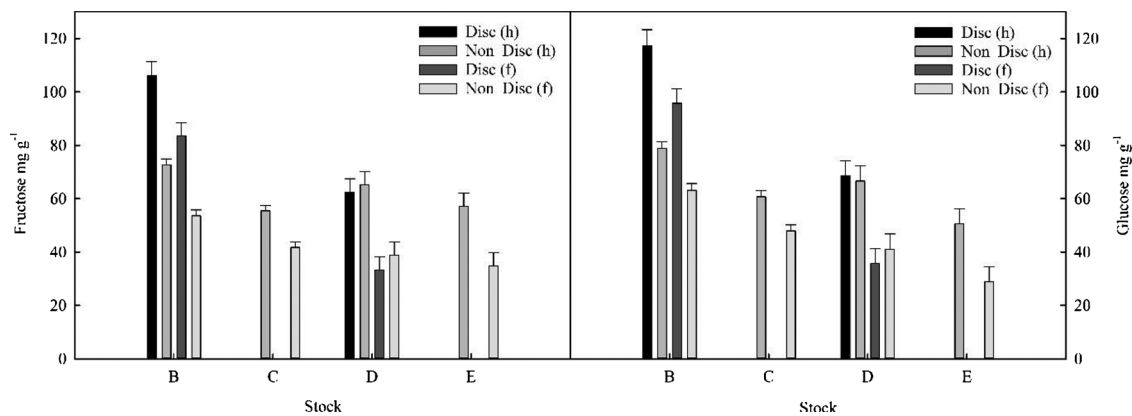


Fig. 6. Fructose and glucose contents (g kg^{-1} DW) in discoloured and non-discoloured heart (h) and flesh (f) samples of season 1 (2011–2012) stocks B (most susceptible) and C (non-susceptible) and season 2 (2012–2013) stocks D (susceptible) and E (non-susceptible).

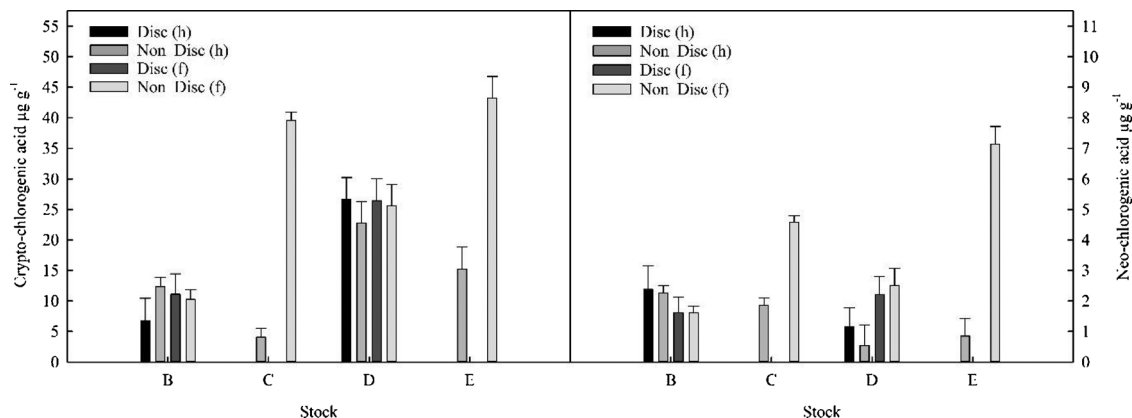


Fig. 7. Crypto- and neo-chlorogenic acids (mg kg^{-1} DW) contents in discolored and non-discolored heart (h) and flesh (f) samples of season 1 (2011–2012) stocks B (most susceptible) and C (non-susceptible) and season 2 (2012–2013) stocks D (susceptible) and E (non-susceptible) according to tissue discoloration regardless the time point.

Previously, it has been argued that tubers affected by other internal physiological disorders, such as brown centre, hollow heart, and internal brown spot, may be misdiagnosed as being BH-affected due to the commonality in symptoms. [Reeve \(1968\)](#) suggested that sometimes these other disorders may overlap or act as precursors for BH induction.

Potato tissue discoloration has been closely linked with phenolic compounds and amino acids accumulation ([Navarre et al., 2009](#)). More specifically, it has been suggested that tyrosine and chlorogenic acid are both adequate substrates for enzymatic oxidation via PPO, resulting in black and brown pigments, respectively. Nevertheless, the relationship between substrate, enzyme and resulting discoloration has yet to be clearly described ([Takahama, 2004](#); [Adams and Brown, 2007](#); [Werij et al., 2007](#)). In the current study, chlorogenic acid and its isomer crypto-chlorogenic acid increased in heart samples of the most susceptible stocks (A and B) during cold storage.

It is well established that low temperature storage can induce higher concentrations of reducing sugars in potato tubers, this being a undesirable trait for the processing industry. The association between reducing sugars and internal disorders is less well documented. [Bussan \(2007\)](#) reported that reducing sugars can accumulate in tubers with brown centre and hollow heart symptoms. [Lipton \(1967\)](#) reported BH incidence in cv. White Rose potato tubers after storage in 0.5–1% O₂ at 15–20 °C with ca. 2-fold lower glucose concentration in the outer and inner parts of the tubers compared to those held in air (21% O₂). The results herein showed that the most susceptible stock (B), showed greater reducing sugar accumulation in both flesh and heart tissues during storage, indicating that BH susceptibility is aligned to non-structural carbohydrate hydrolysis.

5. Conclusion

For the first time targeted metabolomic approaches were performed in order to identify biochemical changes in potato stocks cv. Maris Piper with different susceptibility to BH. From our findings it was hypothesized that tissue discoloration might be initiated during cold storage and be enhanced during shelf-life. Taken together, our results suggest that both the accumulation of reducing sugars and chlorogenic acid isomers could be used as pre-symptomatic biomarkers of BH susceptibility and may allow practitioners to segregate between potato consignments with different propensity to BH during storage.

CRedit authorship contribution statement

Elisavet Kiaitsi: Investigation, Methodology, Formal analysis, Writing - original draft. **Roberta Tosetti:** Formal analysis, Validation, Visualization, Writing - original draft. **Leon A. Terry:** Conceptualization, Funding acquisition, Resources, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.postharvbio.2019.111094>.

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