Food Chemistry xxx (xxxx) xxxx

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Cell wall and metabolite composition of sweet cherry fruits from two cultivars with contrasting susceptibility to surface pitting during storage

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ARTICLE INFO

Keywords:
Prunus avium
Surface pitting
Metabolomic
Cell wall

ABSTRACT

Surface pitting is a serious postharvest physiological disorder in sweet cherries that is observed as skin depressions developed days after bruising. This work aims to compare two cultivars displaying different pitting susceptibilities ('Kordia': relatively resistant; 'Sweetheart': relatively susceptible) using metabolomics profiling and cell wall sugar characterization at different developmental stages and during postharvest storage. Kordia was significantly firmer than Sweetheart, with 1.4-fold more alcohol-insoluble residues (AIRs). A significant correlation was observed between AIRs and deformation, indicating that the highest yields of cell wall material are positively correlated with the resistance to rupture. Additionally, free D-galacturonic acid was higher in pitted Sweetheart samples, likely indicating greater pectin degradation in this susceptible cultivar. Higher contents of the p-coumaric acid derivatives L-5-oxoproline and D-galactose in Sweetheart cherries were found. The metabolic changes during storage and cell wall composition could influence the susceptibility to surface pitting.

1. Introduction

Sweet cherries (*Prunus avium* L.) are highly perishable non-climacteric fruit that are consumed mostly fresh. Additionally, they are a nutrient-dense food with a high concentration of anthocyanins, vitamins and fiber (McCune, Kubota, Stendell-Hollis, & Thomson, 2011). Nevertheless, fruit color, size and defect-free skin are also important attributes to consumers (Chockchaisawasdee, Golding, Vuong, Papoutsis, & Stathopoulos, 2016). Surface pitting is one of the most important physiological disorders that cause damage to sweet cherries (Gonzalez et al., 2016). This disorder has been described as indentations (from 4 to 8 mm) on the surface of the fruit due to the collapse of the cells under the skin. The injury occurs during harvest or different operations in a packing house but develops during storage some days or weeks after bruising (Kappel, Toivonen, Stan, & Mckenzie, 2006; Toivonen, Kappel, Stan, McKenzie, & Hocking, 2004).

More than 1,400 cherry varieties have been described worldwide, but those in commercially important countries such as Chile, the USA, Canada, Australia, Spain and Iran include the following: Bing, Lapins, Sweetheart, Van, Lambert, and Stella, among others (Bujdosó & Hrotkó, 2017). Cultivar differences and preharvest and postharvest factors have been studied in relation to surface pitting susceptibility (Kappel et al., 2006; Kappel, Toivonen, Mckenzie, & Stan, 2002; Toivonen et al., 2004). However, studies relating pitting susceptibility to varieties have shown inconsistent results (Kappel et al., 2006). For example, Sweetheart sweet cherry is one of the most susceptible cultivars to surface pitting in Chile compared with other cultivars such as Kordia, Lapins, Regina and Bing (Param & Zoffoli, 2016; Gonzalez et al., 2016; Espinoza & González, 2015). By contrast, Toivonen et al. (2004) observed higher severity of pitting in Bing than in Sweetheart, both cultivated in Canada. This difference in susceptibility could be due to an interaction between the genotype and environment (Einhorn, Wang, & Turner, 2013). Recently, Param and Zoffoli (2016) reported structural and rheological differences in mesocarp and epidermis cells in sweet

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https://doi.org/10.1016/j.foodchem.2020.128307
Received 18 October 2019; Received in revised form 1 September 2020; Accepted 1 October 2020

cherries. They concluded that a low number of large cells in the external mesocarp area leads to stiffer sweet cherry tissue that leads to lower susceptibility to mechanical damage.

The maturity stage of the fruit is also related to susceptibility to surface pitting, with evidence showing that fruit at an advanced ripeness stage is less susceptible to surface pitting (Toivonen et al., 2004). Nevertheless, Gonzalez et al. (2016) reported that Sweetheart cultivar maturity stages did not affect damage development. Fruit firmness displayed no correlation to surface pitting in Regina cherries, although Sweetheart firmness was positively correlated (Gonzalez et al., 2016). Unpublished results by Einhorn et al. (2013) found a strong positive correlation between fruit firmness and pitting in Sweetheart and Lapins, but the effect is complex and regulated by multiple factors such as maturity, water loss and respiration rate (Toivonen et al., 2004). Therefore, fruit firmness alone is not a good predictor of pitting (Einhorn et al., 2013).

Postharvest factors associated with surface pitting include storage and handling temperature and water loss and/or dehydration (Kappel et al., 2002; Lidster & Tung, 1980; Özkaya et al., 2015; Porrit, Lopatecki, & Meheriuk, 1971; Toivonen et al., 2004). In order to avoid water loss and increase shelf life, the optimum sweet cherry storage conditions have been established at 0 °C with 95% relative humidity (Sen, Oksar, Golkarian, & Yaldiz, 2014). Toivonen et al. (2004) reported that weight loss during storage is a good predictor of pitting susceptibility in the Bing cultivar. However, Gonzalez et al. (2016) established that differences in pitting damage between Regina and Sweetheart cultivars could not be related to fruit weight loss. Regarding temperature, surface pitting has been inversely related to it, i.e. sweet cherries handled or processed on packing lines with lower temperatures displayed more surface pitting (Lidster & Tung, 1980; Porrit et al., 1971).

Sweet cherry surface pitting could be minimized by the reduction of cell wall polysaccharide degradation and suppression of enzymes such as polygalacturonase, pectate lyase and β-D-galactosidase using postharvest hydrogen sulfide fumigation (Zhi & Dong, 2018). Michailidis et al. (2019) recently reported that UV-C-exposed sweet cherries presented a lower incidence of surface pitting due to increased galacturonate, pectin fractions and skin resistance to penetration. However, other postharvest technologies, such as 1-MCP and MAP, did not reduce pitting in cherries during cold storage (Karagiannis et al., 2018). Furthermore, the pitting disorder could induce other changes in the cell. Wang et al. (2019) reported a narrow cell wall of pitted blueberries (30 d of cold storage plus 4 d of shelf life) and wrinkled cell membrane that induced cell plasmolysis.

To our best knowledge, no relationship has been reported between surface pitting disorder and primary and secondary metabolites. Therefore, we hypothesized that sweet cherry cultivars with contrasting pitting susceptibility display differences in phenotypic characteristics, cell wall structure and disassembly patterns and metabolic profiles. We aimed to evaluate the metabolic changes during simulated industrial storage conditions.

2. Materials and methods

2.1. Plant material and sampling

Sweet cherries were harvested on November 2016 in a commercial sweet cherry orchard located in the VI Region of Chile, near Rancagua city. Two cultivars were selected to study pitting: Sweetheart (relatively sensitive) and Kordia (relatively resistant). Four ten-year-old trees per cultivar were randomly selected (n = 4), representing their full production years. Two developmental stages and two postharvest stages were sampled as follows: (S1), straw color stage; (S2), at commercial harvest based on the assessment of skin and flesh color along with total acidity and soluble solids; (S3), after 27 d of cold storage at 1 °C and 95% RH in clamshells; (S4), after 27 d of cold storage at 1 °C and 95% RH plus shelf life at 20 °C for 5 d in clamshells. From each tree, a subsample of 150 fruit at each developmental and postharvest stage was pooled around the canopy representing the whole tree. From these 150 fruits, 50 were used to phenotype and the remaining 100 were used for cell wall characterization, phenolic compound profiling and metabolomics analysis. Each tree was considered as replicate during the entire experiment (n = 4). At S3 and S4, sweet cherries were inspected and separated into two groups—pitted and nonpitted sweet cherries—according to Kappel et al. (2006). No classification on pitting severity was performed.

Whole sweet cherries from each replicate were ground and homogenized in liquid nitrogen in an IKA A11 Basic analytical mill (Sigma Chemical Co., St Louis, MO, USA) and were stored at −80 °C. For primary and secondary metabolite analysis, the samples were freeze-dried. All analyses were performed with whole sweet cherry tissue (skin and flesh).

2.2. Chemicals and reagents

All solvents and reagents were of analytical grade and were purchased from Merck (Darmstadt, Germany). Phenyl-β-glucopyranoside, methoxyamine hydrochloride, N,O-bis(trimethylsilyl)trifluoro-acetamide, pyridine and sugar and phenolic standards were purchased from Sigma Chemical Co. Each reagent and its concentrations are presented in the corresponding relevant section below.

2.3. Phenotyping

2.3.1. Firmness

A penetration test was performed using the TA-XT plus Texture Analyzer (Stable Micro Systems, Godalming, UK) as described by Neven and Drake (2000). Briefly, a 3-mm-diameter cylindrical probe was used to penetrate at 10 mm s^{-1} up to 7 mm in depth. Additionally, complete force–deformation curves were constructed. These curves allowed the determination of the maximum force (N), coefficient of elasticity or gradient (N mm^{-1}), work (mJ), and deformation (%), which, according to various analyses of firmness associated with sensory panels, have demonstrated a high correlation with the parameter of crunchiness (Vargas, Perez, Zoffoli, & Perez, 2001).

2.3.2. Other parameters

Size (mm) and weight (g) were measured for 50 cherries per replicate (n = 4) at each developmental and postharvest stage using a Vernier caliper and an analytic balance, respectively. Next, CIELAB parameters were measured using a Chroma Meter CR-400 (Konica Minolta Sensing Inc., Japan). Finally, soluble solids (%) were measured using a refractometer (Atago Co., Tokyo, Japan) (Param & Zoffoli, 2016).

2.4. Characterization of cell wall sugars

2.4.1. Alcohol-insoluble residue (AIR) preparation

AIRs were obtained as described by Saulnier and Thibault (1987). Sweet cherries (5–10 g) were ground in liquid nitrogen in an IKA A11 Basic analytical mill (Sigma Chemical Co.). Next, 50 mL of 95% ethanol was added, and then the sample was boiled at 80 °C for 10 min. The solution was centrifuged at 6000 *g* for 15 min, and the supernatant was discarded. The solid residue was resuspended in 25 mL of 95% ethanol and vigorously shaken. The suspension was centrifuged at 6000 *g* for 15 min, and the supernatant was discarded. This procedure was performed three times. Finally, the pellet was washed with acetone and dried at room temperature.

2.4.2. Acid hydrolysis

AIR samples (2 mg) were hydrolyzed with 250 μL of 2 M trifluoroacetic acid (TFA) for 1 h at 121 °C. TFA was evaporated under a

stream of nitrogen gas. Next, the samples were washed two times with 250 μL of isopropanol and dried in a Speed-Vac (Eppendorf AG, Germany). The hydrolyzed AIR was suspended in 1 mL of ultrapure water and sonicated for 15 min using an ultrasonic cleaning machine (VWR International, Radnor, PA, USA). The suspension was filtered through a nylon syringe filter with a 0.45-μm pore size.

2.4.3. High-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) for sugar analysis

The separation of sugars from hydrolyzed AIRs was performed using a Dionex DX600 chromatographer equipped with a CarboPac PA1 (4 mm × 50 mm) guard column, two CarboPac Pa1 (4 mm × 250 mm) columns in tandem, and a pulsed amperometric detector. The oven temperature was constant at 26 °C. An isocratic flow of 1 mL min^{-1} of 20 mM NaOH for 20 min was used to separate neutral sugars, followed by a solution of 150 mM sodium acetate and 100 mM NaOH for 15 min for sugar acid separation. Next, a washing step with 200 mM NaOH for 10 min was performed. Quantification of sugars was performed using calibration curves of pure standards (D-fucose, L-rhamnose, L-arabinose, D-galactose, D-glucose, D-xylose, D- mannose, D-galacturonic acid and D-glucuronic acid).

2.5. Analysis of phenolic compounds

For anthocyanin analysis, 0.5 g of freeze-dried sweet cherries were mixed with 15 mL of 80% methanol (containing 0.1% HCl). For phenolic acid extraction, 0.5 g of sample was mixed with 25 mL of 80% methanol. Both extracts were homogenized for 3 min in an orbital shaker and stored at 4 °C for 24 h in the dark. Next, the samples were centrifuged at 10,000 *g* for 10 min at 4 °C. The supernatant was collected and dried in a vacuum at 40 °C. The residue was resuspended in 2.5 mL of 80% methanol (the final concentration of each residue was approximately 1 mg of gallic acid equivalents per mL (for phenolic acid extracts) and 1 mg of cyanidin-3-rutinoside equivalents per mL (for anthocyanin extracts)). The extracts were filtered with a 0.22-μm filter GV type (Millipore) and stored at –80 °C until UPLC-PAD analysis.

Phenolic compounds were analyzed according to Porras-Mija et al. (2020). A UPLC system comprising an Acquity HClass separation module (Waters, Milford, USA) equipped with an autoinjector, an Acquity photodiode array detector (PDA eλ detector) and Empower software was used. The columns used for UPLC separation were the Acquity BEH C18 column (1.7 μm; 100 × 2.1 mm) (Waters, Milford, USA) and Acquity VandGuard BEH C18 precolumn (1.7 μm; 5 × 2.1 mm), operated at 30 °C. Spectral data were recorded from 200 to 700 nm during the whole run. Each extract was analyzed in triplicate. Phenolic compounds were identified and quantified by comparing their retention time and UV–visible spectral data to known previously injected standards. The results were expressed in mg g^{-1} of sample DW.

2.6. GC–MS untargeted metabolome analysis of polar compounds

The extraction and derivatization of polar metabolites were performed according to Fuentealba et al. (2017). Briefly, 20 mg of freeze-dried tissue powder was mixed with cold methanol and phenyl β-D-glucopyranoside as the internal standard. The derivatization comprised methoximation and trimethylsilylation reactions. The samples were analyzed using an Agilent 7890B gas chromatograph equipped with a 5977A single quadrupole MS and an electron impact ionization source (GC–MS), a PAL3 autosampler and an HP-5 ms Ultra Inert (30 m × 0.25 mm × 0.25 μm) column (Agilent Technologies, Santa Clara, CA, USA).

The chromatographic peaks were deconvoluted and identified by comparing retention times and mass spectra to a home-built library of commercial standards and NIST14 library using Mass Hunter Quantitative software (Agilent Technologies, Santa Clara, CA, USA).

2.7. Statistical analysis

The data were subjected to multiway and one-way analysis of variance (ANOVA) with Tukey's test for multiple comparisons ($P < 0.05$) using Statgraphics 18 (StatPoint Inc., Rockville, MD, USA). The results were expressed as means ± standard deviation. The experimental unit was one tree, and four trees were evaluated per cultivar (n = 4). For each replicate, the subsample for cell wall characterization, phenolic compounds and metabolomics analysis was 100 fruits, and for phenotyping 50 fruits were used.

For metabolomics data analysis, principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were performed using Unscrambler® X software (version 10.4; CAMO, Oslo, Norway). The variables were mean centered and weighted by their standard deviations. For PLS-DA, the primary and secondary metabolites were used as predictor variables while the sweet cherry cultivars (Kordia and Sweetheart), pitted (P) and nonpitted samples (N) at S3 and S4 postharvest stages were used as categorical response variables. Important variables were selected using the jack-knife approach, and one-way ANOVA was conducted to determine significant differences in the metabolites among cultivars and development stages. Finally, all primary and secondary metabolites were subjected to metabolic pathway analysis using MetaboAnalyst 4.0 (http://www.metaboanalyst.ca).

3. Results and discussion

3.1. Phenotyping

Firmness in sweet cherries has been extensively studied (Einhorn et al., 2013; Hampson et al., 2014; Sen et al., 2014). In this study, multifactorial ANOVA shows that Kordia sweet cherries are significantly firmer than the Sweetheart cultivar. Differences in the maximum force, work, gradient (coefficient of elasticity) and deformation at the first evaluated developmental stage (S1) were more prominent (Table 1). However, at commercial harvest (S2), after 27 d at 1 °C (S3) and after 27 d at 1 °C plus 5 d at 20 °C (S4), nonsignificant differences were observed in those parameters, except for work (mJ) where the sweet cherries from the Kordia cultivar displayed higher values than those from Sweetheart. During postharvest storage, sweet cherries are exposed to several changes such as cell wall degradation and moisture loss, which result in a decrease in firmness (Correia, Schouten, Silva, & Gonçalves, 2017; Sen et al., 2014). Firmness showed a nonsignificant difference during storage, likely because destructive firmness, which measures the maximum force reached at the skin rupture point (Vargas et al., 2001), was evaluated, unlike the nondestructive method that measures the maximum force at the same penetration distance. Therefore, a variation in distance (mm) was recorded to obtain the deformation parameter. Deformation is the advance distance of the probe until rupture occurs and is expressed as the percentage of the cheek diameter (Vargas et al., 2001). Kordia deformation was significantly higher than that of Sweetheart; in conjunction with the other parameters, this could be interpreted as Kordia sweet cherries being more resistant to rupture. Habib, Bhat, Dar, and Wani (2017) reported the late cultivars of sweet cherry being firmer than early cultivars. However, in our study, Kordia is an early cultivar that was firmer than the late cultivar Sweetheart. Therefore, the differences in firmness between cultivars could be associated with genotypic differences (Esti, Cinquanta, Sinesio, Moneta, & Di Matteo, 2002).

Regarding the size of sweet cherries, both cultivars displayed nonsignificant differences at all development stages. However, Kordia cherries were heavier and had higher soluble solids (%) than Sweetheart at the same developmental stages (Table 1). Serradilla et al. (2012) reported similar contents of soluble solids for the Sweetheart cultivar. However, Kordia had slightly more soluble solids (approximately 1.3-fold) than those reported by Skrzynski, Leja, Gonkiewicz,

Table 1
Quality related parameters of Kordia and Sweetheart sweet cherries at different developmental and postharvest stages. S1: straw color; S2: commercial harvest; S3: storage at 1°C for 27 d; and, S4: storage at 1 °C for 27 d plus shelf life at 20 °C for 5 d.

	Kordia				Sweetheart			
	S1	S2	S3	S4	S1	S2	S3	S4
Soluble solids (%)	9.2 ± 0.4^{a}	22.6 ± 1.2^{c}	23.6 ± 0.9^{cd}	24.8 ± 1.2^{d}	7.4 ± 0.2^{a}	18.6 ± 0.3^{b}	19.5 ± 0.9^{b}	19.7 ± 0.6^{b}
Size parameters								
Weight (g)	4.9 ± 0.4^{b}	12.4 ± 0.2^{f}	11.7 ± 0.6^{ef}	11.0 ± 0.7^{de}	3.6 ± 0.2^{a}	10.6 ± 0.6^{cd}	9.8 ± 0.5^{c}	9.7 ± 0.3^{c}
Groove diameter (mm)	19.1 ± 0.4^{a}	24.9 ± 0.2^{b}	24.3 ± 0.6^{b}	24.0 ± 0.8^{b}	17.6 ± 0.3^{a}	23.8 ± 0.5^{b}	23.6 ± 0.4^{b}	24.8 ± 2.3^{b}
Cheek diameter (mm)	19.9 ± 0.7^{b}	27.8 ± 0.2^{c}	27.7 ± 0.5^{c}	27.3 ± 0.5^{c}	18.4 ± 0.2^{a}	26.7 ± 0.6^{c}	27.7 ± 0.8^{c}	27.7 ± 0.5^{c}
Color parameters								
L*	75.9 ± 0.2^{c}	30.3 ± 2.0^{a}	29.0 ± 0.9^{a}	28.2 ± 0.2^{a}	77.7 ± 0.4^{c}	34.3 ± 0.8^{b}	32.8 ± 0.3^{b}	33.1 ± 0.8^{b}
a*	−10.6 ± 1.1^{a}	17.6 ± 2.1^{c}	17.4 ± 2.7^{c}	13.1 ± 1.9^{b}	−14.5 ± 0.6^{a}	30.6 ± 1.6^{d}	29.0 ± 2.0^{d}	26.8 ± 1.3^{d}
b*	42.3 ± 1.9^{c}	4.4 ± 0.8^{a}	4.2 ± 1.1^{a}	2.7 ± 0.5^{a}	44.4 ± 0.3^{c}	10.9 ± 1.1^{b}	10.2 ± 1.1^{b}	9.5 ± 1.0^{b}
Firmness								
Maximum force (N)	22.0 ± 2.1^{d}	6.2 ± 0.4^{ab}	6.8 ± 0.5^{ab}	7.9 ± 0.7^{b}	19.6 ± 0.5^{c}	5.4 ± 0.3^{a}	6.8 ± 0.4^{ab}	6.7 ± 0.3^{ab}
Gradient (N mm^{-1})	68.3 ± 6.7^{b}	16.1 ± 0.8^{a}	16.2 ± 1.4^{a}	18.8 ± 0.9^{a}	91.0 ± 2.8^{c}	16.5 ± 1.0^{a}	18.2 ± 1.3^{a}	17.1 ± 0.5^{a}
Work (mJ)	3.00 ± 0.26^{e}	1.18 ± 0.10^{ab}	1.40 ± 0.09^{bc}	1.61 ± 0.24^{cd}	1.80 ± 0.12^{d}	0.87 ± 0.06^{a}	1.27 ± 0.04^{bc}	1.26 ± 0.07^{b}
Deformation (%)	15.8 ± 0.2^{e}	13.3 ± 0.6^{bcd}	14.4 ± 0.6^{cde}	15.2 ± 1.7^{de}	10.7 ± 0.6^{a}	11.6 ± 0.5^{ab}	12.9 ± 0.7^{bc}	13.5 ± 0.7^{bcd}

Data are expressed as mean ± standard deviation (n = 4, 50 fruits per replicate). Different letters within a row indicate significant statistical differences ($P < 0.05$).

and Banach (2016) and Usenik, Stampar, Petkovsek, and Kastelec (2015). During storage, increasing contents of soluble solids were detected in both cultivars. However, only Kordia showed a significant increment at the last stage (S4). This increment could be due to water loss, which was also significant at the same stage for this cultivar. At S1, no differences in color were observed between the cultivars. Nevertheless, at harvest and storage stages, Kordia sweet cherries showed a purple and darker color than Sweetheart. Additionally, Kordia at the last stage (S4) showed a significant decrease in parameter a*, which represents the red color. These color parameters agree with those reported by other authors (Saracoglu, Ozturk, Yildiz, & Kucuker, 2017; Usenik et al., 2015).

3.2. Cell wall characterization

The composition of monosaccharides obtained after hydrolysis of cell wall material from sweet cherries is shown in Table 2. The alcohol-insoluble residues (AIRs) of Kordia were 1.4-fold higher than those of Sweetheart, leading to a higher content of several monosaccharides from the cell wall. Salato, Ponce, Raffo, Vicente, and Stortz (2013) reported similar AIR yields from Sweetheart cultivars (1.77%). Additionally, a significant correlation was observed with deformation ($R = 0.82$; $P < 0.01$), showing that higher yields of cell wall material are positively correlated with the sweet cherry resistance to rupture. Kappel et al. (2006) reported that fruit firmness did not have a significant relationship with the severity of surface pitting. In our study, firmness (maximum force) showed a slightly positive correlation with AIR contents ($R = 0.55$; $P < 0.05$). Therefore, the firmer and more relative resistant cultivar to surface pitting (Kordia) positively correlated with its higher contents of AIRs. This result supports the reported by Param and Zoffoli (2016) that firmer fruit with higher contents of AIR exhibit lower surface pitting.

The main neutral sugar from the cell wall for both cultivars corresponded to arabinose. Basanta et al. (2014) reported that this sugar increases its proportion with ripening. Additionally, galactose and rhamnose were quantified in significant amounts, indicating the presence of Ramnogalacturan-I (RG-I) (Basanta et al., 2014; Cao et al., 2018). The presence of glucose and xylose suggests the presence of xyloglucan and xylan (Basanta et al., 2014). These sugars were significantly higher in Kordia than in Sweetheart. Hemicelluloses (e.g. xyloglucan) can form a load-bearing network with cellulose microfibrils.

In addition, arabinan and galactan side-chains of RG-I can bind cellulose microfibrils with similar affinities as xyloglucan, directly contributing to the crosslinking of cellulose microfibrils in the cell wall (Wang, Yeats, Uluisik, Rose, & Seymour, 2018). The firmness in sweet

Table 2
Cell wall monomers (mg g^{-1} AIR) of Kordia and Sweetheart sweet cherries with (P) and without surface pitting (N) at different postharvest stages. S3: storage at 1°C for 27 d; and, S4: storage at 1 °C for 27 d plus shelf life at 20 °C for 5 d.

	Fuc	Rha	Ara	Gal	Glc	Man	Xyl	GalA	GlcA	g AIR 100 g^{-1} FW
Kordia										
S3 – N	3.8 ± 0.7^{aA}	16.3 ± 2.4^{aB}	62.4 ± 4.8^{aB}	34.2 ± 4.3^{aB}	29.7 ± 7.7^{aB}	5.9 ± 0.8^{bA}	8.9 ± 1.4^{aB}	67.9 ± 8.8^{aB}	3.5 ± 0.7^{aB}	2.4 ± 0.6^{aB}
S3 – P	3.7 ± 0.3^{aA}	15.8 ± 2.8^{aB}	58.1 ± 3.5^{aB}	31.3 ± 2.7^{aB}	32.6 ± 8.9^{aB}	5.8 ± 0.5^{bA}	8.6 ± 1.5^{aB}	60.6 ± 10.0^{aB}	3.1 ± 0.6^{aB}	2.3 ± 0.7^{aB}
S4 – N	4.3 ± 0.2^{bA}	16.6 ± 1.8^{aB}	60.5 ± 3.1^{aB}	33.2 ± 1.5^{aB}	33.7 ± 7.3^{aB}	5.4 ± 0.4^{aA}	7.8 ± 0.8^{aB}	62.9 ± 6.4^{aB}	3.3 ± 0.4^{aB}	3.5 ± 0.6^{aB}
S4 – P	4.6 ± 0.9^{bA}	16.0 ± 3.6^{aB}	59.6 ± 6.1^{aB}	31.9 ± 6.2^{aB}	37.5 ± 12.5^{aB}	5.2 ± 1.0^{aA}	7.6 ± 1.9^{aB}	58.6 ± 12.8^{aB}	3.2 ± 0.6^{aB}	2.6 ± 0.3^{aB}
Sweetheart										
S3 – N	3.9 ± 0.6^{aA}	11.9 ± 3.0^{aA}	55.7 ± 5.2^{aA}	30.2 ± 4.1^{aA}	24.8 ± 6.4^{aA}	6.0 ± 0.9^{bA}	7.6 ± 1.5^{aA}	61.7 ± 16.7^{aA}	3.0 ± 0.4^{aA}	1.7 ± 0.3^{aA}
S3 – P	4.0 ± 0.6^{aA}	12.1 ± 3.4^{aA}	56.0 ± 4.6^{aA}	29.6 ± 4.8^{aA}	22.6 ± 3.8^{aA}	5.5 ± 1.0^{bA}	7.2 ± 1.9^{aA}	50.7 ± 22.4^{aA}	2.9 ± 0.4^{aA}	1.9 ± 0.3^{aA}
S4 – N	5.2 ± 0.5^{bA}	10.9 ± 2.2^{aA}	56.6 ± 5.3^{aA}	29.1 ± 4.4^{aA}	23.0 ± 4.5^{aA}	5.0 ± 1.0^{aA}	6.9 ± 2.4^{aA}	42.0 ± 23.4^{aA}	2.4 ± 0.6^{aA}	1.8 ± 0.2^{aA}
S4 – P	4.5 ± 0.7^{bA}	11.5 ± 1.8^{aA}	51.2 ± 2.3^{aA}	26.4 ± 1.5^{aA}	21.8 ± 4.8^{aA}	4.7 ± 0.5^{aA}	5.8 ± 0.8^{aA}	45.5 ± 13.5^{aA}	2.5 ± 0.5^{aA}	2.0 ± 0.2^{aA}

Data are expressed as mean ± standard deviation (n = 4). Different lower-case and upper-case letters within a column stand for significant differences ($P < 0.05$) between stages and cultivars, respectively.

Table 3
Phenolic compounds of Kordia and Sweetheart sweet cherries with surface pitting (P) and without surface pitting (N) at different developmental and postharvest stages. S1: straw color; S2: commercial harvest; S3: storage at 1°C for 27 d; and, S4: storage at 1 °C for 27 d plus shelf life at 20 °C for 5 d.

	Phenolic compounds (mg g^{-1} DW)							
	1	2	3	4	5	6	7	8
Kordia								
S1	0.41 ± 0.04^{c}	ND	28.99 ± 1.69^{d}	ND	ND	2.93 ± 0.14^{d}	26.79 ± 2.12^{d}	2.86 ± 0.20^{c}
S2	Tr	ND	4.05 ± 0.20^{b}	ND	ND	0.55 ± 0.01^{b}	5.20 ± 0.15^{b}	0.70 ± 0.03^{a}
S3 – N	Tr	Tr	3.92 ± 0.22^{b}	ND	ND	0.61 ± 0.03^{b}	4.90 ± 0.41^{b}	ND
S3 – P	ND	ND	3.77 ± 0.14^{b}	ND	0.12 ± 0.00^{b}	0.60 ± 0.04^{b}	4.80 ± 0.04^{b}	ND
S4 – N	ND	ND	3.86 ± 0.38^{b}	ND	0.13 ± 0.01^{b}	0.62 ± 0.06^{b}	4.87 ± 0.40^{b}	ND
S4 – P	ND	ND	3.75 ± 0.33^{b}	ND	0.12 ± 0.00^{b}	0.65 ± 0.05^{b}	4.72 ± 0.04^{b}	ND
Sweetheart								
S1	0.35 ± 0.01^{c}	ND	11.70 ± 0.69^{c}	ND	ND	0.91 ± 0.06^{c}	13.50 ± 1.13^{c}	1.19 ± 0.11^{b}
S2	Tr	0.19 ± 0.02^{b}	1.38 ± 0.09^{a}	0.14 ± 0.02^{b}	0.10 ± 0.01^{a}	0.18 ± 0.01^{a}	2.45 ± 0.24^{a}	ND
S3 – N	0.15 ± 0.01^{b}	0.16 ± 0.02^{ab}	1.18 ± 0.07^{a}	0.11 ± 0.01^{a}	0.11 ± 0.01^{a}	0.20 ± 0.01^{a}	2.53 ± 0.19^{a}	ND
S3 – P	0.11 ± 0.02^{a}	0.17 ± 0.03^{b}	1.22 ± 0.11^{a}	0.13 ± 0.00^{ab}	ND	0.20 ± 0.01^{a}	2.22 ± 0.15^{a}	ND
S4 – N	0.11 ± 0.00^{a}	0.14 ± 0.00^{a}	1.11 ± 0.05^{a}	0.11 ± 0.00^{a}	0.10 ± 0.01^{a}	0.19 ± 0.01^{a}	2.25 ± 0.11^{a}	ND
S4 – P	ND	0.18 ± 0.03^{ab}	1.20 ± 0.13^{a}	0.15 ± 0.02^{b}	ND	0.21 ± 0.02^{a}	2.31 ± 0.10^{a}	ND

	Phenolic compounds (mg g^{-1} DW)						
	9	10	11	12	13	14	Total
Kordia							
S1	0.61 ± 0.04^{d}	1.42 ± 0.03^{c}	0.82 ± 0.08^{b}	1.35 ± 0.27^{bc}	0.40 ± 0.09^{c}	4.83 ± 0.21^{d}	71.44 ± 4.65^{d}
S2	0.18 ± 0.00^{b}	0.21 ± 0.02^{a}	Tr	1.11 ± 0.11^{bc}	0.31 ± 0.09^{bc}	0.98 ± 0.03^{b}	13.29 ± 0.44^{b}
S3 – N	0.19 ± 0.02^{b}	0.20 ± 0.02^{a}	0.10 ± 0.01^{a}	1.05 ± 0.05^{b}	0.23 ± 0.02^{b}	0.85 ± 0.06^{b}	12.06 ± 0.69^{b}
S3 – P	0.19 ± 0.01^{b}	0.20 ± 0.02^{a}	0.14 ± 0.03^{a}	1.14 ± 0.07^{bc}	0.26 ± 0.08^{b}	0.84 ± 0.06^{b}	12.06 ± 0.02^{b}
S4 – N	0.22 ± 0.02^{b}	0.22 ± 0.02^{a}	0.15 ± 0.02^{a}	1.30 ± 0.10^{c}	0.22 ± 0.01^{b}	0.89 ± 0.07^{b}	12.49 ± 0.86^{b}
S4 – P	0.21 ± 0.01^{bc}	0.21 ± 0.03^{a}	0.13 ± 0.01^{a}	1.37 ± 0.12^{c}	0.25 ± 0.03^{b}	0.83 ± 0.07^{b}	12.22 ± 0.81^{b}
Sweetheart							
S1	0.30 ± 0.03^{c}	1.20 ± 0.10^{b}	0.74 ± 0.05^{b}	0.90 ± 0.21^{b}	0.25 ± 0.04^{b}	2.54 ± 0.25^{c}	33.59 ± 2.41^{c}
S2	0.09 ± 0.00^{a}	0.20 ± 0.01^{a}	0.13 ± 0.00^{a}	0.31 ± 0.02^{a}	0.08 ± 0.01^{a}	0.35 ± 0.01^{a}	5.60 ± 0.24^{a}
S3 – N	0.10 ± 0.01^{a}	0.20 ± 0.02^{a}	0.14 ± 0.01^{a}	0.38 ± 0.07^{a}	0.11 ± 0.04^{a}	0.35 ± 0.05^{a}	5.70 ± 0.36^{a}
S3 – P	0.09 ± 0.00^{a}	0.20 ± 0.01^{a}	0.11 ± 0.05^{a}	0.37 ± 0.01^{a}	0.09 ± 0.01^{a}	0.32 ± 0.02^{a}	5.23 ± 0.41^{a}
S4 – N	0.10 ± 0.00^{a}	0.18 ± 0.01^{a}	0.14 ± 0.01^{a}	0.40 ± 0.04^{a}	0.09 ± 0.01^{a}	0.32 ± 0.02^{a}	5.25 ± 0.23^{a}
S4 – P	0.10 ± 0.00^{a}	0.19 ± 0.01^{a}	0.12 ± 0.01^{a}	0.39 ± 0.02^{a}	0.09 ± 0.01^{a}	0.32 ± 0.02^{a}	5.24 ± 0.31^{a}

1–6: p-coumaric acid derivatives (RT: 7.52, 8.02, 9.89, 11.01, 14.95 and 17.09 min); 7–10: chlorogenic acid derivatives (RT: 7.91, 13.65, 19.49 and 21.77 min); 11–13: rutin derivatives (RT: 18.33, 20.32 and 21.31 min); and, 14: chlorogenic acid (RT: 10.70 min). Data are expressed as mean ± standard deviation (n = 4). Different letters within a column indicate significant statistical differences ($P < 0.05$). Tr: traces; and ND: not detected.

cherries is largely determined by covalently-bound pectins and matrix glycans (Belge, Comabella, Graell, & Lara, 2015), which is consistent with the results of this study.

By contrast, fucose and mannose were the lowest neutral sugars found and showed no significant differences between the cultivars. Salato et al., 2013 (within Gonzalez et al., 2016) reported that a higher content of cell wall material, less branching of tightly bound pectins in the cell wall and a lower content of neutral sugar-rich pectin side chains are present in firmer cultivars. Finally, galacturonic and glucuronic acid in Kordia sweet cherries were higher than those in Sweetheart, likely contributing to surface pitting resistance in the Kordia cultivar. Skin resistance to the penetration of sweet cherry was positively correlated with the total pectin level, and galacturonic acid is the main component of pectins (Michailidis et al., 2019). Xin, Chen, Lai, and Yang (2017) reported that most of sodium carbonate-soluble pectins form large aggregates in cherries. After cold storage the structure of pectins changes, where increasing cleavage points, short chains and depolymerization can be observed mainly catalyzed by pectinesterases and polygalacturonases (Belge et al., 2015; Xin et al., 2017; Zhi & Dong, 2018).

The differences in cell wall compositions between Kordia and Sweetheart cultivars during cold storage correlated with firmness and its relative resistance to mechanical damage. Despite the differences between cultivars, no differences were found between pitted and nonpitted sweet cherries. This phenomenon could be due to the lack of compositional changes during surface pitting, and larger amounts of cell wall material might improve the resistance of sweet cherries (deformation) and, therefore, the surface pitting resistance.

3.3. Phenolic compounds

Phenolic compounds are shown in Table 3. The total phenolic content was in the range of 5.23 and 71.44 mg g^{-1} (DW) and was significantly higher in Kordia than Sweetheart. The three main compounds were p-coumaric acid derivative (RT = 9.89 min), chlorogenic acid derivative (RT = 7.91 min), and chlorogenic acid (RT = 10.70 min) (compounds 3, 7 and 14 in Table 3, respectively). The first compound could correspond to p-coumaroyl quinic acid, which has been reported by several authors (Gonçalves et al., 2004; Mozetič, Trebše, Simčič, & Hribar, 2004; Serrano et al., 2009). Additionally, neochlorogenic acid is an abundant phenolic compound reported in sweet cherries. Likely, the chlorogenic acid derivative (RT = 7.91) detected in our study corresponds to this compound. The content of most of the phenolics was significantly higher at the first developmental stage (S1). During the ripening of sweet cherries, an increase in fruit weight and soluble solids content occurs, and phenolic compounds such as chlorogenic acid transform into anthocyanins (Correia et al., 2017; Habib et al., 2017). No differences in phenolics during postharvest in each cultivar were found. Similar results were reported by Sen et al. (2014), who indicated that sweet cherries harvested at optimum maturity and stored in cold and room temperature did not undergo significant changes in the content of total phenols and antioxidant capacity. Multiway ANOVA showed no differences between pitted and nonpitted sweet cherries, except for a p-coumaric acid derivative (RT = 11.01, compound 4 in Table 3) in the cultivar Sweetheart after 27 d at 1 °C plus 5 d at 20 °C (S4) evaluated with one-way ANOVA. In this case, Sweetheart sweet cherries with surface pitting presented higher contents of this phenolic acid than nonpitted samples (0.15 ± 0.02 and 0.11 ± 0.0 mg g^{-1} (DW), respectively). Nevertheless, these contents were low compared with the total phenolic content.

Cyanidin-3-rutinoside is the main anthocyanin found in both cultivars and was significantly higher in Kordia than Sweetheart sweet cherries (4.72–6.60 and 1.45–2.29 mg g^{-1} DW, respectively). These values agree with those reported by Gonçalves et al. (2004). Cyanidin-3-glucoside was present in traces in Sweetheart and the range of 0.34–0.76 mg g^{-1} DW in Kordia (Table 4). The contents of anthocyanin are correlated with the color of sweet cherries (Habib et al., 2017); in the case of cyanidin-3-rutinoside, the correlations with the color parameters L*, a* and b* (R = −0.88, R = −0.96 and R = −0.96, respectively) were significant ($P < 0.01$). The contents of all anthocyanins were higher in the last two stages (S3 and S4). Similar results were obtained by Gonçalves et al. (2004) and Serrano et al. (2009), where cold storage induced the synthesis of anthocyanins in ripe and partially ripe cherries. The main factors that affect the concentration of phenolic compounds and anthocyanins are the type of cultivar and storage conditions (Habib et al., 2017). However, no differences were observed between pitted and nonpitted sweet cherries in Kordia and Sweetheart. In all pitted samples, the total anthocyanin content was slightly higher than that in nonpitted samples ($P > 0.05$). Only for Sweetheart after 27 d at 1 °C (S3), the pitted sweet cherries showed higher contents of cyanidin-3-rutinoside than nonpitted samples (2.14 and 1.45 mg g^{-1} DW, respectively). Bunsiri, Ketsa, and Paull (2003) reported phenolic acid synthesized after a mechanical impact in mangosteen (*Garnicia mangostana* L.). Secondary metabolites may act as antiradicals neutralizing cherry skin tissue damage by oxidative stress originated by cold storage (Michailidis et al., 2019). To our best knowledge, this is the first report of phenolic compounds being evaluated in pitted sweet cherries. However, further studies are needed to

Table 4
Anthocyanin contents of Kordia and Sweetheart sweet cherries with surface pitting (P) and without surface pitting (N) at different postharvest stages. S2: commercial harvest; S3: storage at 1°C for 27 d; and, S4: storage at 1 °C for 27 d plus shelf life at 20 °C for 5 d.

	Anthocyanins (mg g^{-1} DW)				
	Cyanidin-3-glucoside	Cyanidin-3-rutinoside	Unknown 1*	Unknown 2*	Total
Kordia					
S2	0.41 ± 0.10^{ab}	4.72 ± 0.39^{a}	0.01 ± 0.01^{ab}	0.05 ± 0.03^{abc}	5.20 ± 0.52^{b}
S3 – N	0.34 ± 0.11^{a}	4.95 ± 0.49^{a}	0.01 ± 0.01^{b}	0.04 ± 0.02^{a}	5.34 ± 0.61^{b}
S3 – P	0.39 ± 0.11^{ab}	5.11 ± 0.74^{bc}	0.02 ± 0.01^{bc}	0.05 ± 0.03^{abc}	5.57 ± 0.88^{b}
S4 – N	0.65 ± 0.17^{bc}	6.46 ± 0.47 ^{cd}	0.03 ± 0.01^{c}	0.13 ± 0.03^{c}	7.26 ± 0.66^{c}
S4 – P	0.76 ± 0.24^{c}	6.60 ± 0.71^{d}	0.03 ± 0.01^{c}	0.12 ± 0.05^{bc}	7.51 ± 1.01^{c}
Sweetheart					
S2	Tr	1.89 ± 0.34^{a}	Tr	0.05 ± 0.02^{ab}	1.94 ± 0.36^{a}
S3 – N	Tr	1.45 ± 0.90^{a}	Tr	0.03 ± 0.02^{a}	1.47 ± 0.92^{a}
S3 – P	Tr	2.14 ± 0.22^{a}	Tr	0.08 ± 0.01^{abc}	2.22 ± 0.23^{a}
S4 – N	Tr	2.12 ± 0.26^{a}	Tr	0.09 ± 0.03^{abc}	2.21 ± 0.27^{a}
S4 – P	Tr	2.29 ± 0.24^{a}	Tr	0.13 ± 0.04^{bc}	2.42 ± 0.27^{a}

*Quantified as cyanidin-3-rutinoside. Data are expressed as mean ± standard deviation (n = 4). Different letters within a column indicate significant statistical differences ($P < 0.05$). Tr: traces.

ascertain whether mechanical stress induces the synthesis of cyanidin-3-rutinoside in Sweetheart fruit after cold storage.

3.4. GC–MS untargeted metabolome analysis of polar compounds

Polar metabolites detected by GC–MS include organic acids, amino acids and sugars, among others. Fig. 1 shows principal component analysis performed on those compounds. PCA extracted 5 PCs, which explained 74.1% of the total variability. The first component discriminated between developmental stages and the second between cultivars. However, the other 3 PCs showed no clear difference between samples (data not shown). One-way ANOVA ($P < 0.05$) of metabolites showing greater variability (compounds that are further from the origin in Fig. 1) was performed. Regarding the primary metabolites identified,

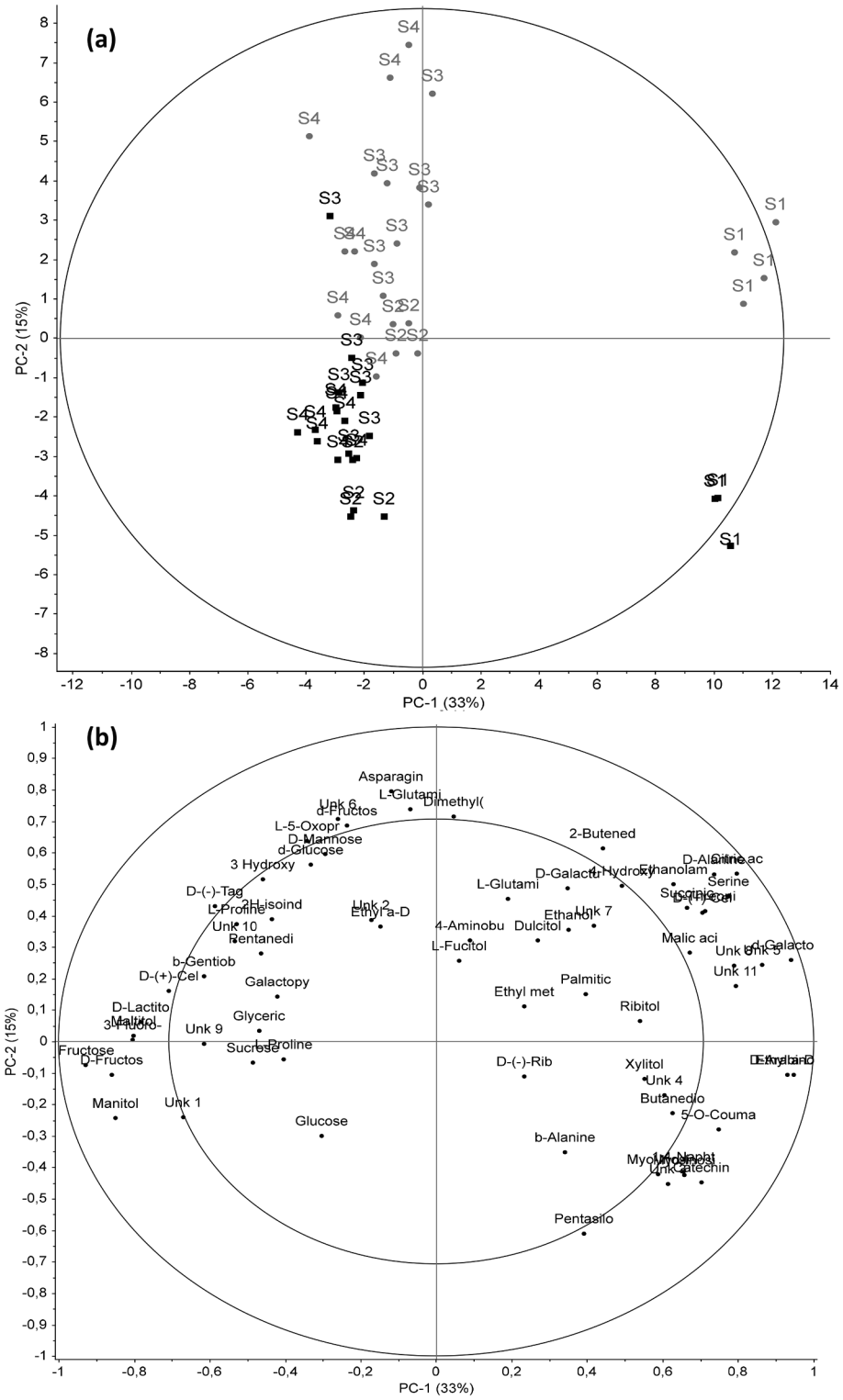

Fig. 1. Principal component analysis (PCA) for GC–MS of untargeted metabolites in sweet cherries. (a) Score plot and (b) loading plot. The Kordia cultivar is represented in black color, and Sweetheart is represented in gray color. S1: straw color; S2: commercial harvest; S3: storage at 1˚C for 27 d; S4: storage at 1 ˚C for 27 d plus shelf life at 20 ˚C for 5 d. One hundred fruits were used per replicate (n = 4).

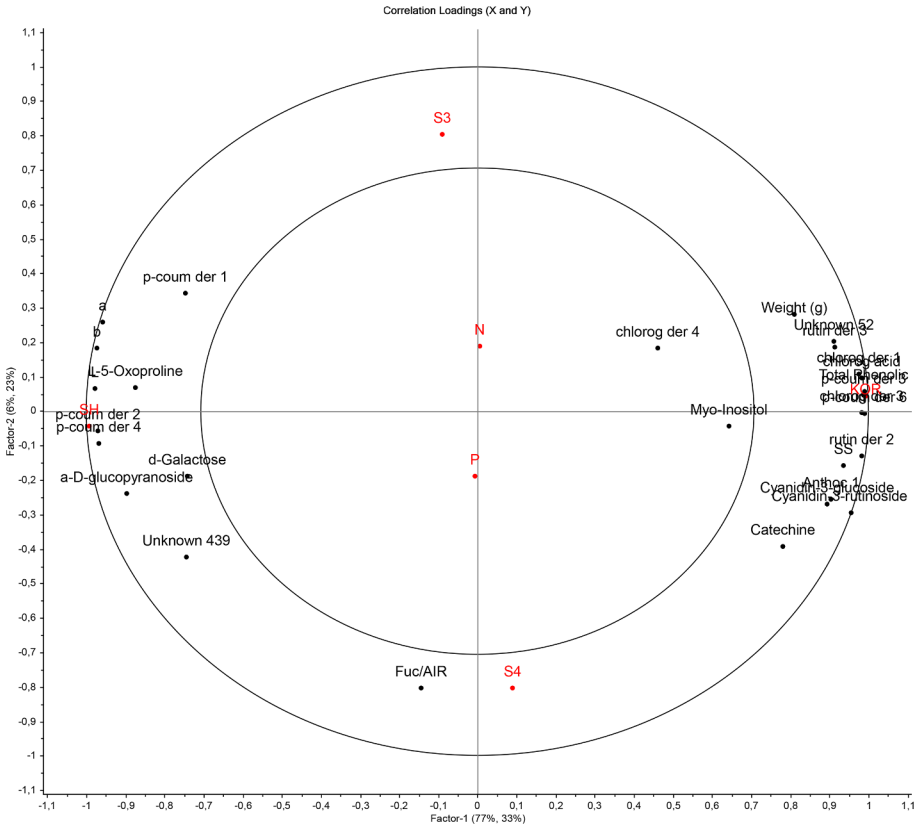

Fig. 2. Partial least squares with discriminant analysis (PLS-DA) biplot for the main primary and secondary metabolites of contrasting phenotypes of sweet cherries. The predictor variables are represented by black dots, and the categorical response variables are represented by red dots. Kor: Kordia cultivar; SH: Sweetheart cultivar; P: pitted samples; nonpitted samples; S3: storage at 1°C for 27 d; and, S4: storage at 1 °C for 27 d plus shelf life at 20 °C for 5 d. One hundred fruits were used per replicate (n = 4). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Sweetheart sweet cherries at the straw color stage (S1) showed higher contents of organic acids (such as citric, succinic and malic acid) and amino acids such as L-alanine and serine than Kordia cultivar at S1 (Figure supplementary 1). These compounds are involved in the citrate cycle and biosynthesis of several amino acids, which sustain several metabolic pathways (KEGG, 2020). The pathway analysis showed a higher impact in the aminoacyl-tRNA biosynthesis ($P < 0.05$) in Sweetheart samples at S1 and S2, and lower impact in the inositol phosphate metabolism at S1 rather than Kordia. At S2, glyoxylate, dicarboxylate, butanoate, propanoate, alanine, aspartate and glutamate metabolism showed a higher impact in both sweet cherries varieties. However, cold storage and pitted samples did not show significant changes in metabolic pathways. Cultivar differences in organic acids and sugars during ripening have been reported by other authors (Serradilla et al., 2012; Skrzynski et al., 2016). Additionally, Sweetheart samples at commercial maturity and cold stored had higher contents of individual compounds such as D-mannose, asparagine, L-glutamate and L-5-oxoproline than Kordia. Glucose, fructose and sucrose are the main sugars reported in sweet cherries (Özkaya et al., 2015). However, no significant differences were found in those sugars between the cultivars. The D-mannose content increased after cold storage (S3) and then decreased after 5 days at room temperature (S4) for both cultivars. However, in both stages, the D-mannose content was higher for Sweetheart than Kordia. This monosaccharide is involved in fructose, mannose and galactose metabolism (KEGG, 2020). Brizzolara, Manganaris, Fotopoulos, Watkins, and Tonutti (2020) reviewed the influence of cold storage on carbohydrate metabolism in fruit such as citrus, nectarines and peaches, where higher contents of sucrose and sugar alcohols may contribute to membrane stability. Amino acid metabolism can also be affected by low-temperature storage (Karagiannis et al., 2018). However, contrasting results were found after cold storage for amino acids such as asparagine, L-glutamate and 4-aminobutiric acid (GABA), which are involved in alanine, aspartate, and glutamate metabolism (KEGG, 2020). GABA is an important intermediate of nitrogen metabolism that may act as an osmoprotectant under stress conditions and balance a rapid decrease in carbohydrates (Brizzolara et al., 2020). Finally, L-5-oxoproline and L-glutamate are involved in glutathione metabolism, indicating that the antioxidant protection mechanism could occur in Sweetheart sweet cherries after cold storage, especially in the pitted samples (KEGG, 2020; Mirto et al., 2018).

Discrimination between pitted and nonpitted samples was not possible by PCA. However, D-galacturonic acid, the main component of pectin, was higher in pitted Sweetheart samples at S4. Thus, more degradation of pectins occurred in this cultivar susceptible to surface pitting (Zhi & Dong, 2018), a finding that is consistent with the decreased D-galacturonic acid found in AIR (Table 2).

To investigate the relationship among all variables studied (primary and secondary metabolites, cell wall monosaccharides and quality variables) and differences among the cultivars, postharvest stages and pitted samples, PLS-DA was conducted. Because the straw color stage in both cultivars was significantly different in most parameters, it was not considered for this multivariate analysis. Moreover, important variables with a P-value < 0.05 were selected for PLS-DA. Fig. 2 shows that the main categorical discrimination occurs between cultivars due to the phenolic composition. The sweet cherries cv. Kordia had higher contents of anthocyanins, which confer a dark purple color. Additionally, these samples were larger and had higher contents of soluble solids and myo-inositol, which play a biological role in the synthesis of sugar alcohols (KEGG, 2020). However, Sweetheart showed higher contents of some p-coumaric acid derivatives, L-5-oxiproline and D-Galactose. As stated above, carbohydrate metabolism could be altered during cold storage and the extent differs among varieties (Brizzolara et al., 2020). Moreover, D-galactose is a major branching monosaccharide of RG-I that is present in its free form likely due to pectin solubilization during storage (S3 and S4). Salato et al. (2013) reported that RG-I is likely the polyuronide preferentially solubilized in Sweetheart sweet cherries.

However, the fucose concentration in AIRs was significantly higher in the last stage of storage (S4) than S3, with nonsignificant differences

between the cultivars. Fucose is a neutral monosaccharide found in hemicellulose (Basanta et al., 2014) that could increase its proportion in AIRs while other monosaccharides are decreased, such as galacturonic acid, xylose and mannose (Table 2).

No discrimination between pitted sweet cherries and nonpitted samples was observed in PLS-DA. In this study, the variability observed was strongly driven by the cultivars. However, the differences between these contrasting cultivars in pitting allow us to understand that differences in metabolic changes could influence the susceptibility to surface pitting.

4. Conclusions

Differences in primary and secondary metabolites were observed between both sweet cherry cultivars. The 'Sweetheart' cultivar susceptible to surface pitting showed higher contents of the p-coumaric acid derivatives L-5-oxiproline, D-galactose and D-galacturonic acid. The latter compound was significantly higher in pitted samples after cold storage and shelf-life. Several metabolic pathways—e.g., glutathione and carbohydrate metabolism related—were increased in Sweetheart samples. The composition of free and cell wall monosaccharides showed that pectin solubilization could occur more intensely in Sweetheart samples after storage. Additionally, higher contents of cell wall material were positively correlated with resistance to rupture (deformation), explaining the relative resistance to surface pitting of Kordia sweet cherries. This study shows, for the first time, the metabolic changes in sweet cherries during development and cold storage and potential involvement in the development of surface pitting. However, more analyses, especially related to cell wall structure, are needed to understand the response to mechanical stress in both cultivars. Understanding of surface pitting on sweet cherries would allow the development of treatments based on molecular knowledge of the disorder.

CRediT authorship contribution statement

Claudia Fuentealba: Methodology, Formal analysis, Investigation, Writing - review & editing, Writing - original draft. **Troy Ejsmentewicz:** Formal analysis, Investigation. **Reinaldo Campos-Vargas:** Conceptualization, Methodology, Supervision. **Sebastian Saa:** Methodology. **Oscar Aliaga:** Resources. **Rosana Chirinos:** Investigation. **David Campos:** Investigation, Writing - review & editing. **Romina Pedreschi:** Conceptualization, Methodology, Writing - review & editing, Supervision.

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.

Acknowledgements

This study was supported by Conicyt Fondecyt 11170360, Conicyt Fondequip EQM140074 and Conicyt PCI REDBIO0001 (Chile) grants.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2020.128307.

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