



Effects of hot water treatments on the main physicochemical characteristics and the levels of vitamin C and polyphenols of two sweet cherry cultivars (*Prunus avium* L.) during cold storage and shelf-life

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Abstract

The possible effects of hot water treatments (HWT) on the main quality parameters and bioactive compounds of two sweet cherry cultivars were examined over two years. Cherries were dipped in hot water (48 °C, 2 min), stored 3 weeks at 1 °C (CS) and for 2 additional days at 20 °C for simulated shelf-life (SL). Except for a slight decrease in firmness generally observed in treated samples (5–6%), no difference was observed between HWT and control fruits, neither for the main quality parameters nor the bioactive compounds during CS and/or SL. On average, the percentage of rotten fruit was more than 50% lower in HWT samples compared to the controls. These results show that the HWT conditions examined in this study effectively reduce the incidence of sweet cherry decay without impairing, after CS and/or SL, most of the main quality parameters or the content of the main bioactive nutrients. Because HWT are residue-free, easy to apply and effective in reducing post-harvest losses while preserving the organoleptic and nutritional quality of cherries, HWT are a good strategy for both cherry professionals and consumers.

Keywords Sweet cherry · Hot water treatment · Cold storage · Shelf-life · Polyphenols

Introduction

With a global production increase of about 50% between 2001 and 2021, 1.8 and 2.7 million tons, respectively [1], sweet cherry (*Prunus avium* L.) is a very popular fruit worldwide with a high market value. Highly appreciated by consumers for its attractive color and sweetness, sweet cherry is also known to be a good source of health beneficial compounds, such as vitamins and phenolics [2–5]. In particular, sweet cherries have been reported to exhibit potential cancer chemopreventive properties, or to reduce the risk of developing diabetes and Alzheimer's disease [6–8]. In recent years, consumers have become increasingly aware of the impact of food on health, and many of them are willing to pay higher prices for fresher, tastier, and healthier

products [9]. As a non-climacteric fruit, sweet cherry must be picked when fully ripe to guarantee good quality [10], but due to its short postharvest shelf-life, cold storage is commonly used to extend its market window. Nevertheless, as the storage time increases fruit quality generally significantly decreases (water loss, softening, ...) and postharvest rots, mainly caused by fungal pathogens (*Monilinia* spp., *Botrytis cinerea*, *Penicillium expansum*, ...) often occur [11, 12]. Although these fungal pathogens can be controlled using synthetic fungicides, there is a growing demand from consumers and regulatory authorities to decrease, or even prohibit, their use [13]. Pre-storage hot water treatments (HWT) have previously been reported to be effective and natural alternatives to synthetic chemical treatments against postharvest rots in many fruits including cherries [14–17].

Nevertheless, information on the influence of HWT on nutritional and bioactive compounds is scarce, and apart from some work [18–22], little is known about their potential impacts on polyphenolic compounds. Therefore, the objective of this study was to determine over two years the effects of HWT on the levels of vitamin C and polyphenols

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in two sweet cherry cultivars after 3 weeks of cold storage at 1 °C and after an additional shelf-life period of 2 d at 20 °C. Because firmness, sweetness and acidity are decisive characteristics for consumer acceptance of sweet cherries [23], fruit firmness, soluble solids content, titratable acidity as well as levels of individual sugars and organic acids were determined. The efficiency of HWT on fruit rot was also evaluated.

Materials and methods

Chemicals and reagents

All chemicals and reagents were as previously described [24] unless otherwise indicated.

Materials

In 2020 and 2021, two sweet cherry (*Prunus avium* L.) cultivars, Balrine and Regina, were manually harvested at commercial ripening stage in the experimental orchard of the Interprofessional Technical Center for Fruit and Vegetables (CTIFL) of Balandran (Gard, France), and then transferred to the laboratory within two hours. For each year and cultivar, about 6–8 kg of homogeneous fruits in color and size and without any visual defects were selected. Fruits were then randomly packed into commercial punnets of 250 g. Three punnets of 25 fruits were then randomly selected and directly analyzed at harvest as described below. Remaining punnets were then randomly divided into two groups: one group was the untreated control, whereas the other was dipped into hot water at 48 °C for 2 min (HWT). These conditions were chosen based on results obtained in preliminary experiments carried out at the CTIFL (data not shown). Punnets were left for 2 h at room temperature until the water had evaporated, and then punnets of control and HWT fruits were stored for 3 weeks at 1 °C (90% RH). At the end of cold storage (CS), an additional shelf-life period of 2 d at 20 °C (SL) was applied. Three punnets of control and HWT fruits were analyzed at the end of CS, and at the end of SL. Each punnet was considered as a biological replicate.

Fruit decay evaluation

Fruit rot was visually evaluated at the end of CS and SL. Fruit that showed any sign of surface mycelia development were considered as decayed. Rot ratio was expressed as a percentage of infected sweet cherry fruits.

Firmness

Firmness was performed on both sides of each fruit from each replicate using an electronic Durofel (licensed by CTIFL-Copa Technologie, Saint Etienne du Grès, France) fitted with a 0.25 mm tip. The measurements, ranging from 0 (no resistance) to 100 (maximum resistance), are expressed in ID₂₅. Fruits were then hand-pitted, cut into small cubes (< 1 cm³) and frozen under liquid nitrogen. The cubes were then immediately reduced to powder using an IKA A11 analytical mill for 30 s (IKA, Staufen, Germany) with liquid nitrogen, and total vitamin C was immediately determined as described below (see “Vitamin C” section). The frozen powders were then stored at – 80 °C until analysis.

Soluble solids content (SSC) and titratable acidity (TA)

About 20 g of frozen powder (– 80 °C) were then thawed for 30 min at room temperature and then centrifuged (14,000g, 5 min, 4 °C) (Sigma 4K15, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). SSC was determined from the supernatant with an Atago PR-32 digital refractometer (Atago Co., Ltd., Tokyo, Japan). TA was determined by diluting 5 mL of supernatant with 30 mL of deionized water, and titrating to pH 8.1 with 0.1 N NaOH using an automatic titrator with autosampler (Titroline 7000, Schott SI Analytics, Mainz, Germany).

Individual sugars and organic acids

The levels of sugars and organic acids were simultaneously determined by HPLC as previously described [24]. Briefly, 1 mL of supernatant previously obtained, diluted 20-fold with deionized water, was filtered (RC 0.2 µm Phenex; Phenomenex, Le Pecq, France) and directly injected into the HPLC system. Identifications were performed by comparing retention times (t_R) with those of standards and by spiking samples with pure compounds.

Vitamin C

Total vitamin C was determined by HPLC as previously described [24]. Briefly, 1 g of frozen powder added to 10 mL of metaphosphoric acid (MPA) (2%) was homogenized for 60 s at 20,000 rpm with an Ultra-Turrax (IKA T25-Digital). After centrifugation (Sigma 4K15, 14,000g, 5 min, 4 °C), the supernatant was filtered (RC 0.2 µm Phenex; Phenomenex). Total vitamin C was determined by HPLC after 3 h of reduction under agitation at room temperature of 500 µL of supernatant added to 500 µL of tris-(2-carboxy-ethyl)-phosphine

TCEP (0.01 mol/L) in MPA (2%). Chromatographic conditions were as previously described. Identifications were performed by comparing retention times (t_R) with that of the standard of ascorbic acid and by spiking samples with a pure compound.

Polyphenols

The levels of polyphenols were determined by UPLC-MS as previously described [24]. Briefly, four grams of frozen powder added with 20 mL of a mixture of MeOH/H₂O/formic acid (60/38/2 v/v/v) and 100 μ L of methyl 4-hydroxybenzoate (3515 mg/L in MeOH/formic acid; 95/5 v/v) (internal standard) were homogenized for 60 s at 20,000 rpm with an Ultra-Turrax (IKA T25-Digital) and centrifuged (Sigma 4K15, 10,000g, 5 min, 4 °C). 10 mL of supernatant was concentrated to dryness under a stream of nitrogen using an XcelVap automated evaporation system (Horizon technology, New Hampshire, USA). The residue was dissolved in 1 mL of mixture of MeOH/formic acid (95/5 v/v), filtered on Phenex RC 0.2 μ m (Phenomenex) and then injected in UPLC-MS. The UPLC-MS analyses were performed on an ACQUITY UPLC system equipped with a photo-diode array (PDA) detector (Waters, Milford, MA, USA) coupled to a mass single-quadrupole detector (QDa, Waters), which is a compact detector with an electrospray ionization (ESI) interface. Empower 3 software was used for data acquisition and instrument control. Separation was carried out at 35 °C using a Waters Acquity HSS T3 column (100 mm \times 2.1 mm i.d., 1.8 μ m) protected by a precolumn filter (Waters). A binary solvent system was used at a 0.4 mL/min flow rate with solvent A (water/formic acid; 98.5:1.5 v/v) and solvent B (methanol). The elution gradient was as follows: 0–1 min, isocratic 0% B; 1–18 min, linear 0–74.5% B; 18–22 min, isocratic 95% B; 22–27 min, isocratic 0% B. The volume of injection was 1 μ L. With the PDA detector, the flavonols were detected at 255 nm, flavanols at 280 nm, hydroxycinnamic acids at 320 nm, and anthocyanins at 505 nm. Compound identification was achieved by comparing the retention times (t_R) and their UV–Vis spectra from 240 to 600 nm with those of standards, and by spiking samples with pure compounds whenever possible. The QDa detector was operated in negative and positive ion modes for polyphenol and anthocyanin characterization. ESI capillary voltage and cone voltage were set at 0.8 kV and 15V, respectively. Probe temperature was set at 600 °C. A full mass spectrum between m/z 100 and 1200 was acquired at a sampling rate of 8.0 points/sec. Flavonols were quantified by comparisons with an external standard of quercetin-3-glucoside, flavan-3-ols as (+)-catechin, hydroxycinnamic acid derivatives as 5-caffeyolquinic acid, and anthocyanins as cyanidin-3-glucoside. Levels of phenolic compounds were expressed as milligrams per 100 g of FW.

Statistical analysis

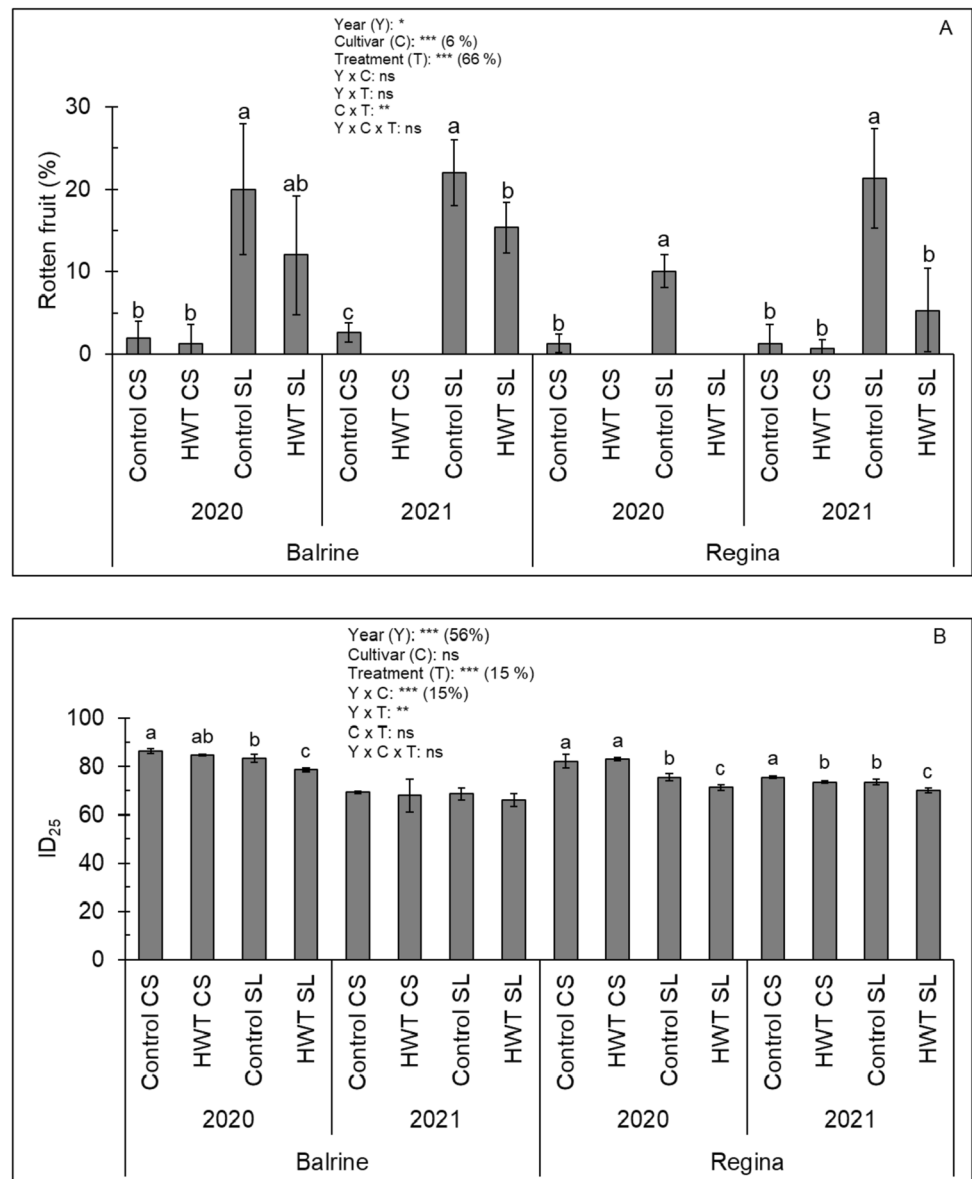
Data are given as mean \pm standard deviation ($n=3$). Significant differences between the five treatments (Harvest, Control CS, HWT CS, Control SL, and HWT SL) were evaluated year by year for each cultivar through one-way analysis of variance (ANOVA). Homogeneity of variance was tested using Levene's median test. Welch-ANOVA was applied when data were not homoscedastic. Multiple comparisons of means were carried out using Duncan's test ($p < 0.05$). Data were also analyzed by three-way ANOVA using a completely randomized design (2 years \times 2 cultivars \times 5 treatments \times 3 replicates). The effect size of the different factors and their interaction was evaluated using omega squared value (ω^2) calculated as follow: $\omega^2 = (SS_{\text{Factor}} - df_{\text{Factor}} \times MS_{\text{Error}}) / (SS_{\text{Total}} + MS_{\text{Error}})$, where SS_{Factor} = sum of squares of each factor, df_{Factor} = degrees of freedom of each factor, MS_{Error} = mean square error and SS_{Total} = sum of squares total [25]. The obtained value, multiplied by 100, thus represents the variance in the population as explained by each factor. As compared to eta squared and partial eta squared, ω^2 was preferred because this estimation resulted in less bias when dealing with small samples. Statistical analyses of data were performed using XLSTAT 2019.1.1 (Addinsoft, Paris, France).

Results and discussion

Fruit decay and firmness

Three-way ANOVA results (Fig. 1A) showed that the percentage of rotten fruit was highly affected ($p < 0.001$) by the postharvest treatment and the cultivar. According to the omega-squared value (ω^2), which explains the contribution of each factor to the observed variability, the postharvest treatment was the most significant factor ($\omega^2 = 66\%$), followed by cultivar ($\omega^2 = 6\%$). Year ($p < 0.05$) and the interaction cultivar \times treatment ($p < 0.01$) also affected the percentage of rotten fruit, but the ω^2 values were below 5%. No significant interaction year \times cultivar or treatment, or year \times cultivar \times treatment was observed. As shown in Fig. 1A, HWT notably reduced the percentage of rotten fruit as compared to untreated samples, particularly after the two additional days of shelf-life at 20 °C. Over the two years and for the two cultivars, the percentage of rotten fruit in HWT samples (8%) was on average more than half that in the controls (18%). Lastly, as indicated Fig. 1B, flesh firmness was mainly affected ($p < 0.001$) by the year ($\omega^2 = 56\%$), followed by the postharvest treatment ($\omega^2 = 15\%$) and the interaction year \times cultivar ($\omega^2 = 15\%$). The interaction year \times treatment ($p < 0.01$) also affected the firmness but the ω^2 values was below 5%. Flesh firmness was not influenced by cultivar.

Fig. 1 Percentage of rotten fruits (A) and firmness (B) in control and HWT sweet cherries after 21 d of cold storage at 1 °C (CS) and additional 2 d of shelf-life at 20 °C (SL) in 2020 and 2021. Values are the mean \pm standard deviation ($n=3$). For a given year and cultivar, values with different letters are significantly different ($p<0.05$, Duncan's test). For three-way ANOVA results, only $\omega^2>5\%$ are indicated in parenthesis. ns (not significant); * ($p<0.05$); ** ($p<0.01$); *** ($p<0.001$)



No significant interaction cultivar \times treatment or cultivar \times year \times treatment was observed. As observed, at the end of CS, flesh firmness of fruit was generally similar between samples, except for Regina in 2021, where the flesh firmness of HWT fruit was about 3% lower than in controls. As previously reported [26], firmness of heated fruits were after SL less firm (5–6%) compared to control ones, excepted for Balrine in 2021 where no difference was observed.

Soluble solids content (SSC) and titratable acidity (TA)

As observed in Table 1, SSC ranged from 16.4 to 19.9°Brix in the different sweet cherry samples and TA from 8.5 to 13.5 meq/100g. Three-way ANOVA results showed that SSC was mainly affected ($p<0.001$) by the year ($\omega^2=42\%$),

the cultivar ($\omega^2=24\%$) and the interaction year \times cultivar ($\omega^2=18\%$). As shown, neither the treatment factor nor its interaction with year and/or cultivar significantly affected SSC. In contrast, TA was mainly affected ($p<0.001$) by the postharvest treatment ($\omega^2=45\%$), followed by the year ($\omega^2=24\%$), the interaction year \times cultivar ($\omega^2=17\%$) and the cultivar ($\omega^2=5\%$). No interaction treatment with year and/or cultivar was observed for TA. For both years and both cultivars (Table 1), SSC remained constant in control fruits during the 21 d of cold storage at 1 °C (CS) and/or after the two additional days of shelf-life at 20 °C (SL), whereas TA decreased significantly by about 16–23% during CS and then remained constant during SL. These results agree with those previously reported in other sweet cherry cultivars, and confirm that SSC is generally not affected by cold storage whereas a loss of acidity is observed [27–29]. As shown

Table 1 Soluble solids content (SSC), titratable acidity (TA), levels of sugars, malic acid and vitamin C in control and HWT sweet cherries after 21 d of cold storage at 1 °C (CS) and additional 2 d of shelf-life at 20 °C (SL) in 2020 and 2021

Year (Y)	Cultivar (C)	Treatment (T)	SSC (°Brix)	TA (meq/100 g FW)	Sugars (g/100 g FW)			Total	Malic acid (g/100 g FW)	Vitamin C (mg/100 g FW)		
					Glucose	Fructose	Sorbitol				Sucrose	
2020	Baltrine	Harvest	17.0±0.4	13.2±0.7 a	6.8±0.2	5.6±0.2	2.6±0.1	0.10±0.01 b	15.2±0.5	1.2±0.0 a	11.5±0.8 a	
		Control CS	16.6±0.2	10.2±0.4 b	6.8±0.2	5.7±0.2	2.5±0.1	0.13±0.01 a	0.13±0.01 a	15.1±0.2	1.0±0.0 b	8.7±0.4 c
		HWT CS	17.1±0.3	10.7±0.3 b	6.9±0.2	5.8±0.1	2.6±0.1	0.13±0.01 a	0.13±0.01 a	15.4±0.3	1.0±0.0 b	10.1±0.5 b
		Control SL	16.4±0.4	9.9±0.2 b	6.7±0.2	5.6±0.2	2.5±0.1	0.12±0.01 a	0.12±0.01 a	14.9±0.5	0.9±0.0 c	7.8±0.7 cd
		HWT SL	16.9±0.1	10.4±0.2 b	7.0±0.1	5.9±0.2	2.5±0.1	0.09±0.01 b	0.09±0.01 b	15.6±0.3	1.0±0.0 b	7.6±0.4 d
	Regina	P	ns	***	ns	ns	ns	***	ns	ns	***	***
		Harvest	16.7±0.4	10.4±0.3 a	7.3±0.3	5.7±0.2	2.0±0.1	0.11±0.01 c	0.11±0.01 c	15.1±0.6	1.1±0.0 a	15.8±0.6 a
		Control CS	17.2±0.1	8.8±0.2 b	7.4±0.1	5.9±0.1	2.1±0.1	0.15±0.01 a	0.15±0.01 a	15.5±0.2	1.0±0.1 b	10.6±0.3 b
		HWT CS	17.0±0.6	9.0±0.5 b	7.5±0.4	5.9±0.2	2.1±0.2	0.11±0.01 c	0.11±0.01 c	15.6±0.7	0.9±0.0 b	10.6±0.7 b
		Control SL	17.0±0.5	8.7±0.2 b	7.3±0.3	5.8±0.2	2.0±0.2	0.14±0.01 b	0.14±0.01 b	15.2±0.7	0.9±0.0 b	9.7±0.6 b
2021	Baltrine	HWT SL	16.9±0.2	8.5±0.1 b	7.3±0.1	6.1±0.0	2.0±0.1	0.13±0.01 b	0.13±0.01 b	15.5±0.1	0.9±0.0 b	9.8±0.2 b
		P	ns	***	ns	ns	ns	ns	***	ns	***	***
		Harvest	17.4±0.2	13.0±0.7 a	7.6±0.1	5.8±0.1	3.1±0.0	0.15±0.01 c	0.15±0.01 c	16.7±0.2	1.2±0.1 a	10.0±0.5 a
		Control CS	17.0±1.0	10.8±0.4 b	7.4±0.5	5.8±0.4	2.9±0.3	0.20±0.02 ab	0.20±0.02 ab	16.3±1.1	1.0±0.0 b	8.4±0.7 b
		HWT CS	17.9±0.9	10.7±0.4 b	7.7±0.3	6.1±0.3	3.2±0.2	0.22±0.04 a	0.22±0.04 a	17.2±0.8	1.0±0.0 b	8.0±0.6 b
	Regina	Control SL	17.2±0.2	10.4±0.4 b	7.3±0.1	5.9±0.1	2.9±0.1	0.19±0.01 abc	0.19±0.01 abc	16.2±0.3	1.0±0.1 b	6.5±0.1 c
		HWT SL	17.1±0.2	10.5±0.5 b	7.2±0.2	5.8±0.2	3.0±0.2	0.17±0.03 bc	0.17±0.03 bc	16.2±0.5	1.0±0.1 b	6.6±0.2 c
		P	ns	***	ns	ns	ns	*	*	ns	***	***
		Harvest	19.9±0.3	13.5±0.4 a	9.1±0.2	6.5±0.2	3.4±0.1	0.19±0.01	0.19±0.01	19.2±0.5	1.3±0.0 a	13.0±0.7 a
		Control CS	19.2±0.7	11.2±0.6 b	8.7±0.2	6.5±0.2	3.1±0.2	0.21±0.02	0.21±0.02	18.5±0.6	1.1±0.0 b	9.4±0.8 bc
Three-way ANOVA p value (ω ²)	C	HWT CS	19.2±0.7	11.0±0.4 b	8.6±0.4	6.4±0.3	3.0±0.2	0.21±0.04	0.21±0.04	18.2±0.9	1.1±0.0 b	9.9±0.4 b
		Control SL	19.6±0.4	11.1±0.4 b	8.8±0.3	6.8±0.3	3.1±0.2	0.19±0.01	0.19±0.01	18.9±0.7	1.1±0.0 b	7.9±0.3 d
		HWT SL	19.6±0.4	11.2±0.1 b	8.7±0.2	6.7±0.2	3.1±0.2	0.21±0.03	0.21±0.03	18.8±0.5	1.1±0.0 b	8.4±0.4 cd
		P	ns	***	ns	ns	ns	ns	ns	ns	***	***
		Y	*** (42)	*** (24)	*** (45)	*** (29)	*** (74)	*** (69)	*** (31)	*** (10)	*** (10)	*** (10)
	Y	C	*** (24)	*** (5)	*** (36)	*** (29)	***	**	*** (15)	***	*** (21)	*** (21)
		T	ns	*** (45)	ns	ns	ns	*** (8)	ns	*** (46)	*** (58)	*** (58)
		Y×C	*** (18)	*** (17)	*** (7)	*** (12)	*** (11)	ns	*** (11)	*** (12)	ns	ns
		Y×T	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
		C×T	ns	ns	ns	ns	ns	*	ns	ns	ns	***
Y×C×T	Y×C×T	ns	ns	ns	ns	ns	ns	ns	ns	ns	*	

Values are the mean ± standard deviation (n = 3). For a given year and cultivar, values with different letters are significantly different (*p* < 0.05, Duncan's test). For three-way ANOVA results, only ω² > 5% are indicated in parenthesis. ns (not significant); * (*p* < 0.05); ** (*p* < 0.01); *** (*p* < 0.001)

in Table 1, no significant difference was observed between HWT and control fruits for the SSC and TA values, either after CS and/or 2 d of SL.

Sugars and organic acids

In this study, the quantified sugars were glucose, fructose, sorbitol and sucrose, on average 46%, 37%, 16% and 1% respectively of the total sugars, that ranged between 14.9 and 19.2 g/100g FW in the different samples (Table 1). Three-way ANOVA results showed that total sugars were highly affected by the year ($\omega^2=60\%$), the cultivar ($\omega^2=15\%$), and the interaction year \times cultivar ($\omega^2=11\%$). Similar trends were observed for glucose and fructose, while sorbitol and sucrose were mainly affected by the year ($\omega^2=69\text{--}74\%$). With levels ranging from 0.9 to 1.2 g/100g FW (Table 1), malic acid was, as previously reported in other sweet cherry cultivars [5, 30], the main organic acid detected in Balrine and Regina cherries. Citric and shikimic acids were also detected in this study, but at trace levels, and therefore not quantified. Three-way ANOVA results showed malic acid, was mainly affected ($p<0.001$) by the postharvest treatment ($\omega^2=46\%$), followed by the year ($\omega^2=31\%$), the interaction year \times cultivar ($\omega^2=12\%$) and the cultivar ($\omega^2=2\%$). No interaction treatment with year and/or cultivar was observed. For both years and both cultivars (Table 1), the levels of the three main sugars remained constant in control fruit during CS and/or after SL, whereas those of malic acid decreased significantly by about 12–18% during CS and then remained constant during SL. Consistent with results of SSC and TA, these results are also in agreement with those previously reported in other sweet cherry cultivars [31, 32].

As shown in Table 1, no significant difference was observed between HWT and control fruits for the levels of glucose, fructose, sorbitol and malic acid, either after CS and/or SL.

Vitamin C

In this study, vitamin C contents in the different sweet cherry samples ranged between 6.5 and 15.8 mg/100g FW (Table 1). Three-way ANOVA results showed vitamin C was mainly affected ($p<0.001$) by the postharvest treatment ($\omega^2=58\%$), followed by the cultivar ($\omega^2=21\%$), and the year ($\omega^2=10\%$). No interaction year \times cultivar or year \times treatment was observed. Significant interaction cultivar \times treatment ($p<0.001$) and year \times cultivar \times treatment ($p<0.05$) were observed but the ω^2 values were lower than 5%. For both years and both cultivars, levels of vitamin C in control fruits significantly decreased from between 16 and 33% after CS, and then decreased again from between 8 and 22% after SL. These results are in agreement with those previously reported in other sweet cherry cultivars, and confirm that

vitamin C drastically decreases during cold storage and/or shelf-life [29, 33, 34]. As shown in Table 1, a similar trend was observed in HWT fruits. Whether after CS and/or SL, levels of vitamin C in treated and untreated fruits were generally not statistically different, except for Balrine where the decrease observed in HWT fruits after CS in 2020 was half of that observed in control fruits (12% and 24%, respectively).

Polyphenols

In this study, six hydroxycinnamic acid derivatives (HA1–HA6), four flavan-3-ols (FA1–FA4), three anthocyanins (AN1–AN3) and three flavonols (FO1–FO3) were identified and quantified using UPLC-PDA-QDa (Table 2). To our knowledge, this is the first time that a detailed quantitative analysis of individual phenolic compounds is reported in the Balrine sweet cherry cultivar. As previously reported in other cultivars [2, 35–37], hydroxycinnamic acids were predominant (32–50% of the total polyphenol content), followed by anthocyanins (21–42%), flavan-3-ols (20–29%), and flavonols (3–4%) (Table 3). Among hydroxycinnamic acids, neochlorogenic acid (HA1; 17.0–27.4mg/100g FW), 3-p-coumaroylquinic acid (HA2; 10.5–17.9mg/100g FW), and chlorogenic acid (HA3; 3.2–5.3 mg/100g FW) were predominant and together accounted for 93–96% of total hydroxycinnamic acids. Three-way ANOVA results indicated that the levels of the three main hydroxycinnamic acids were significantly ($p<0.001$) affected by year and cultivar. According to ω^2 value, the year explained 65–73% of the total variability of neo and chlorogenic acid, while the cultivar explained 65% of the total variance of 3-p-coumaroylquinic acid. As indicated in Table 3, the levels of the three main hydroxycinnamic acids were not affected by the treatment factor. No interaction was observed for neochlorogenic acid. A significant year \times cultivar interaction ($p<0.001$) was observed for 3-p-coumaroylquinic acid and chlorogenic acid but the ω^2 values were below 5%. Remaining interactions, year \times treatment for 3-p-coumaroylquinic acid ($p<0.05$) or cultivar \times treatment for chlorogenic acid ($p<0.05$), were also below 5%.

Previous studies reported that cold storage has variable effects on phenolic acids in sweet cherries, depending mainly on cultivar and storage conditions [38]. While a decrease of neochlorogenic acid and 3-p-coumaroylquinic acid was observed after a month at 4 °C in the sweet cherries of Bing cultivar [39], their levels were found to remain relatively constant in the cultivars Van and Burlat after 30 d of storage but were found to decrease in the cultivars Saco and Summit during the same period of time [2]. Similarly, while the levels of these two compounds were not affected in the Lambert Compact cultivar after 12 d of storage at 2–4 °C [40], their levels sharply increased in the Sweetheart

Table 2 Retention time, UV_{vis} and MS data of polyphenols in sweet cherry samples

Code	Compound	Rt (min)	UV_{max} (nm)	MS ^a (m/z)
<i>Hydroxycinnamic acids</i>				
HA1	Neochlorogenic acid ^b	5.9	325	353
HA2	3-p-Coumaroylquinic acid	6.8	310	337
HA3	Chlorogenic acid ^b	7.5	325	353
HA4	4-Caffeoylquinic acid ^b	7.9	325	353
HA5	4-p-Coumaroylquinic acid	8.8	311	337
HA6	di-Caffeoylquinic acid	11.3	327	515
<i>Flavan-3-ols</i>				
FA1	Procyanidin dimer B1 ^b	6.2	278	577
FA2	(+)-Catechin ^b	6.8	278	289
FA3	Procyanidin dimer B2 ^b	7.4	278	577
FA4	(-)-Epicatechin ^b	8.4	278	289
<i>Anthocyanins</i>				
AN1	Cyanidin 3-O-glucoside ^b	9.2	280, 515	449
AN2	Cyanidin 3-O-rutinoside ^b	9.6	280, 593	595
AN3	Peonidin 3-O-rutinoside	10.5	280, 520	609
<i>Flavonols</i>				
FO1	Quercetin triglycoside	10.3	265, 347	771
FO2	Quercetin 3-O-rutinoside ^b	11.7	255, 355	609
FO3	Kaempferol 3-O-rutinoside	12.8	265, 348	593

^aMS data in negative mode except for anthocyanins in positive mode

^bIdentified according to a commercial standard

cultivar after 27 d at 0 °C [41]. In our study, for both years and both cultivars (Table 3), no significant change was observed in control fruits for the levels of the three main hydroxycinnamic acids after CS and/or after SL. Similar results were observed in HWT fruits. No significant difference was observed between HWT and control fruits for the levels of the three main hydroxycinnamic acids, either after CS and/or SL.

Cyanidin-3-rutinoside (AN2; 16.9–51.7 mg/100g FW) was the major anthocyanin detected in this study and accounted for 88–94% of total anthocyanins (Table 3). Three-way ANOVA results showed cyanidin-3-rutinoside was mainly affected ($p < 0.001$) by the cultivar ($\omega^2 = 77\%$) and the postharvest treatment ($\omega^2 = 10\%$). Cyanidin-3-rutinoside was also affected ($p < 0.001$) by the year and the interaction year \times cultivar but the ω^2 values were below 5%. No significant interaction between treatment with year and/or cultivar was observed. As shown in Table 3, for both years and both cultivars, levels of cyanidin-3-rutinoside significantly increased in control fruits by about 3–18% during CS, and then increased again by about 19–31% after subsequent SL. These results agree with those previously reported in other cherry cultivars [2, 40, 42, 43]. The accumulation of anthocyanins during storage and/or subsequent SL is attributed to normal sweet cherry ripening, as has been found in other fruits [24, 44, 45]. A similar trend was observed in HWT fruits, and, whether after CS and/or SL, no significant

difference was observed between treated and untreated samples.

With ω^2 values ranging from 54 to 67%, three-way ANOVA results showed that the cultivar was the most relevant factor ($p < 0.001$) in explaining the variability of the four flavan-3-ols detected in this study (Table 3). Except for (+)-catechin (FA2), the year factor also significantly ($p < 0.001$) accounted for a proportion of variance for three of the four flavan-3-ols, ranging from 25% for procyanidin dimer B2 (FA3) to 34% for procyanidin dimer B1 (FA1). As indicated, levels of flavan-3-ols were not affected by the treatment factor. A significant ($p < 0.01$) year \times cultivar interaction was observed for (+)-catechin but the ω^2 value was less than 5%. Remaining interactions, year \times cultivar for procyanidin dimer B1 ($p < 0.05$) and (-)-epicatechin ($p < 0.05$), cultivar \times treatment for procyanidin dimer B2 ($p < 0.05$) and (-)-epicatechin ($p < 0.05$) were all less than 5%.

For a given year and cultivar (Table 3), no significant difference was observed for the levels of the four flavan-3-ols between the control and HWT samples, whether after CS and/or SL.

Among the three flavonols detected in this study, quercetin 3-O-rutinoside (FO2) was predominant (62–71% of total flavonols) (Table 3). Three-way ANOVA results showed that quercetin 3-O-rutinoside and quercetin triglycoside (FO1) were mainly affected ($p < 0.001$) by the cultivar ($\omega^2 = 62$ –81%). The year factor significantly

Table 3 Levels of polyphenols (mg/100 g FW) in control and HWT sweet cherries after 21 d of cold storage at 1 °C (CS) and additional 2 d of shelf-life at 20 °C (SL) in 2020 and 2021

Year (Y)	Cultivar (C)	Treatment (T)	Hydroxycinnamic acids									Anthocyanins				Total
			HA1	HA2	HA3	HA4	HA5	HA6	Total	AN1	AN2	AN3				
2020	Balrine	Harvest	22.9±1.2	14.1±0.2	4.5±0.3	0.6±0.1	0.4±0.0 ab	0.9±0.1	43.4±1.6	1.2±0.1 c	16.9±0.7 c	0.5±0.1 c	18.6±0.8 c			
		Control CS	24.7±1.7	14.1±0.5	4.6±0.3	0.7±0.1	0.5±0.0 a	1.0±0.0	45.5±2.2	0.9±0.1 d	19.9±1.4 b	0.7±0.1 b	21.5±1.5 b			
		HWT CS	22.7±1.3	13.4±0.4	4.1±0.2	0.6±0.1	0.4±0.0 b	1.0±0.0	42.1±2.0	1.1±0.2 cd	19.5±1.2 b	0.7±0.1 b	21.2±1.3 b			
		Control SL	23.8±1.3	13.4±0.5	4.4±0.4	0.7±0.1	0.4±0.0 b	1.0±0.0	43.6±1.5	1.7±0.1 b	24.2±0.9 a	1.1±0.0 a	26.9±1.0 a			
		HWT SL	24.0±1.0	14.0±0.5	4.3±0.2	0.6±0.1	0.4±0.0 b	1.1±0.1	44.4±1.3	2.4±0.2 a	25.0±0.5 a	1.1±0.0 a	28.4±0.7 a			
	Regima	<i>p</i>	ns	ns	ns	ns	*	ns	ns	***	***	***	***			
		Harvest	26.8±0.7	17.9±0.6	5.3±0.5	0.8±0.0	0.4±0.0	0.8±0.1	52.0±1.4	1.6±0.5	31.0±5.2 c	0.9±0.2 c	33.5±5.8 b			
		Control CS	25.6±0.5	16.4±1.0	4.9±0.3	0.8±0.0	0.3±0.0	0.8±0.1	48.8±1.7	1.7±0.3	36.1±2.3 abc	1.3±0.2 ab	39.1±2.4 ab			
		HWT CS	27.4±2.3	17.5±0.6	5.3±0.3	0.8±0.0	0.3±0.0	0.9±0.1	52.3±3.0	1.6±0.4	33.8±7.4 bc	1.0±0.2 bc	36.4±8.0 b			
		Control SL	25.7±2.6	16.0±0.4	4.9±0.2	0.8±0.0	0.3±0.0	0.8±0.0	48.6±2.1	1.9±0.5	43.4±3.5 a	1.5±0.1 a	46.8±4.0 a			
2021	Balrine	HWT SL	25.9±1.2	17.6±1.3	5.4±0.2	0.8±0.1	0.3±0.0	0.9±0.0	51.0±2.5	2.5±0.2	41.9±0.4 ab	1.6±0.1 a	46.0±0.4 a			
		<i>p</i>	ns	ns	ns	ns	ns	ns	ns	ns	*	***	*			
		Harvest	17.4±0.2	11.0±0.2	3.5±0.1	0.4±0.0	0.4±0.0	1.5±0.2	34.2±0.5	0.8±0.1 b	19.5±1.7 b	0.6±0.1 c	20.9±1.9 c			
		Control CS	17.1±2.1	10.9±1.2	3.3±0.4	0.4±0.1	0.4±0.0	1.4±0.1	33.5±3.8	0.8±0.2 b	20.1±3.3 b	0.7±0.0 bc	21.6±3.5 c			
		HWT CS	17.7±0.3	10.7±0.2	3.2±0.2	0.5±0.0	0.4±0.0	1.5±0.1	34.0±0.5	0.8±0.2 b	21.4±3.0 b	0.6±0.2 bc	22.8±3.4 bc			
	Regima	Control SL	17.0±1.2	10.6±0.2	3.4±0.2	0.4±0.1	0.3±0.0	1.5±0.0	33.2±1.5	2.1±0.3 a	26.5±1.5 a	1.1±0.2 a	29.7±1.9 a			
		HWT SL	17.6±1.0	10.5±0.6	3.3±0.2	0.5±0.0	0.4±0.0	1.4±0.1	33.5±2.0	2.2±0.3 a	23.9±1.5 ab	0.9±0.1 ab	27.0±1.7 ab			
		<i>p</i>	ns	ns	ns	ns	ns	ns	ns	***	*	***	***			
		Harvest	20.1±0.2	14.4±0.3	3.4±0.0	0.5±0.0	0.4±0.0	0.8±0.0	39.6±0.1	1.5±0.1 b	40.6±1.8 b	1.3±0.1 b	43.3±1.8 b			
		Control CS	22.0±1.7	15.5±0.8	3.6±0.3	0.6±0.0	0.4±0.0	0.9±0.1	42.8±1.7	1.5±0.2 b	43.6±2.7 b	1.4±0.2 b	46.5±2.9 b			
Three-way ANOVA <i>p</i> value (α^2)	Balrine	HWT CS	21.5±0.7	15.8±0.7	3.6±0.2	0.6±0.0	0.4±0.0	0.9±0.0	42.8±1.1	1.5±0.1 b	41.8±1.3 b	1.2±0.1 b	44.5±1.4 b			
		Control SL	21.1±1.0	15.5±0.7	3.8±0.1	0.6±0.0	0.4±0.0	0.9±0.0	42.1±1.9	2.6±0.4 a	51.7±2.1 a	1.7±0.1 a	56.0±2.5 a			
		HWT SL	21.1±1.3	15.4±1.0	3.7±0.1	0.6±0.0	0.4±0.0	0.9±0.1	42.1±2.4	2.7±0.1 a	50.1±1.2 a	1.7±0.0 a	54.4±1.2 a			
		<i>p</i>	ns	ns	ns	ns	ns	ns	ns	***	***	***	***			
		Y	*** (65)	*** (25)	*** (73)	*** (57)	ns	*** (20)	*** (55)	ns	***	***	***			
	Regima	C	*** (21)	*** (65)	*** (12)	*** (30)	*** (15)	*** (49)	*** (35)	*** (16)	*** (77)	*** (54)	*** (75)			
		T	ns	ns	ns	*	ns	ns	ns	*** (64)	*** (10)	*** (32)	*** (13)			
		Y×C	ns	***	***	ns	*** (34)	*** (24)	ns	ns	***	***	***			
		Y×T	ns	*	ns	ns	*** (8)	ns	ns	**	ns	*	ns			
		C×T	ns	ns	*	ns	*	ns	ns	ns	ns	ns	ns			
2020	Balrine	Y×C×T	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns			
		Harvest	1.9±0.2	3.1±0.5	5.2±0.1	11.8±0.3	22.0±1.0	0.4±0.0 ab	2.1±0.3 bc	0.7±0.3	3.2±0.6	87.2±2.3 b				
		Control CS	2.1±0.4	2.8±0.7	5.7±0.3	12.7±0.8	23.3±2.1	0.4±0.0 a	2.2±0.2 abc	0.7±0.1	3.3±0.3	93.5±4.8 ab				
		HWT CS	1.7±0.1	3.2±0.5	5.1±0.2	11.4±0.7	21.5±1.3	0.4±0.0 b	1.8±0.1 c	0.4±0.1	2.6±0.1	87.5±4.7 b				
		Treatment (T)	FA1	FA2	FA3	FA4	Total	FO1	FO2	FO3	Total					
	Cultivar (C)	Harvest	1.9±0.2	3.1±0.5	5.2±0.1	11.8±0.3	22.0±1.0	0.4±0.0 ab	2.1±0.3 bc	0.7±0.3	3.2±0.6	87.2±2.3 b				
		Control CS	2.1±0.4	2.8±0.7	5.7±0.3	12.7±0.8	23.3±2.1	0.4±0.0 a	2.2±0.2 abc	0.7±0.1	3.3±0.3	93.5±4.8 ab				
		HWT CS	1.7±0.1	3.2±0.5	5.1±0.2	11.4±0.7	21.5±1.3	0.4±0.0 b	1.8±0.1 c	0.4±0.1	2.6±0.1	87.5±4.7 b				

Table 3 (continued)

Year (Y)	Cultivar (C)	Treatment (T)	Flavan-3-ols				Flavonols				Total polyphenols		
			FA1	FA2	FA3	FA4	Total	FO1	FO2	FO3	Total	FO3	FO2
2021	Regina	Control SL	1.8±0.2	3.0±0.2	5.7±0.3	12.0±0.6	22.5±0.9	0.4±0.0 b	2.6±0.3 a	0.8±0.3	3.8±0.6	96.8±3.3 a	
		HWT SL	1.7±0.2	3.4±0.3	5.7±0.5	11.8±0.8	22.6±1.8	0.4±0.0 b	2.3±0.1 ab	0.5±0.1	3.2±0.2	98.6±1.6 a	
		<i>p</i>	ns	ns	ns	ns	ns	*	*	ns	ns	**	
		Harvest	2.6±0.1	5.7±0.1	6.7±0.4	19.6±1.2	34.6±1.6	0.9±0.1	2.6±0.4	0.5±0.2	4.1±0.5	124.1±4.0	
		Control CS	2.5±0.0	6.1±0.8	6.7±0.1	18.7±0.4	34.1±0.9	0.9±0.1	3.0±0.3	0.6±0.1	4.5±0.5	126.5±3.7	
		HWT CS	2.6±0.2	7.1±1.8	7.5±0.2	20.6±1.3	37.7±0.8	1.0±0.0	3.0±0.4	0.7±0.3	4.7±0.8	131.1±11.4	
	Baltrine	Control SL	2.4±0.1	7.5±1.7	7.4±0.8	18.6±0.8	35.9±0.5	0.8±0.0	3.0±0.3	0.5±0.1	4.3±0.4	135.6±4.5	
		HWT SL	2.5±0.1	7.1±1.6	7.6±0.9	19.1±1.0	36.2±2.2	1.0±0.0	3.3±0.1	0.6±0.1	4.9±0.3	138.0±4.4	
		<i>p</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
		Harvest	1.2±0.1	3.9±0.7	4.0±0.3	8.0±0.3	17.0±1.4	0.6±0.0	2.0±0.1	0.5±0.1	3.1±0.1	75.1±1.2	
		Control CS	1.1±0.2	4.0±0.7	4.3±0.3	8.0±0.6	17.4±1.7	0.7±0.1	1.8±0.3	0.4±0.1	2.8±0.5	75.3±8.4	
		HWT CS	1.1±0.0	4.0±0.2	4.1±0.4	7.9±0.7	17.1±1.0	0.6±0.0	1.9±0.3	0.4±0.1	3.0±0.4	76.8±3.6	
Regina	Control SL	1.1±0.0	3.4±0.3	4.6±0.3	7.6±0.2	16.7±0.4	0.7±0.0	2.2±0.2	0.4±0.1	3.2±0.3	82.8±2.8		
	HWT SL	1.1±0.1	3.4±0.5	4.3±0.0	7.7±0.3	16.5±0.3	0.6±0.1	2.2±0.1	0.5±0.0	3.4±0.1	80.4±3.9		
	<i>p</i>	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		
	Harvest	2.0±0.1	4.7±0.1	5.9±0.2	13.8±0.4	26.5±0.7	1.0±0.0	2.7±0.1 b	0.6±0.1	4.3±0.1	113.6±2.5 c		
	Control CS	2.1±0.1	5.5±0.4	5.9±0.2	14.1±0.7	27.5±1.0	1.1±0.0	2.7±0.4 b	0.6±0.1	4.3±0.5	121.1±4.8 b		
	HWT CS	2.2±0.3	6.6±1.4	6.4±0.3	14.9±0.7	30.1±0.2	1.1±0.1	2.7±0.4 b	0.6±0.2	4.3±0.6	121.7±2.2 b		
Three-way ANOVA <i>p</i> value (ω^2)	Control SL	1.9±0.2	6.6±2.3	6.2±0.3	14.5±0.8	29.1±3.6	1.1±0.1	3.2±0.1 a	0.5±0.0	4.8±0.1	132.1±4.9 a		
	HWT SL	1.8±0.2	5.6±0.4	6.2±0.4	14.4±0.6	28.0±1.4	1.2±0.1	3.2±0.2 a	0.5±0.1	4.9±0.4	129.4±4.5 a		
	<i>p</i>	ns	ns	ns	ns	ns	ns	*	ns	ns	**		
	Y	*** (34)	ns	*** (25)	*** (29)	*** (20)	*** (14)	*	* (8)	ns	*** (6)		
	C	*** (54)	*** (64)	*** (61)	*** (67)	*** (74)	*** (81)	*** (62)	ns	*** (69)	*** (85)		
	T	ns	ns	ns	ns	ns	ns	*** (10)	ns	*	***		
Y×C	Y×C	*	**	ns	*	**	*	ns	** (9)	ns	**		
	Y×T	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		
	C×T	ns	ns	*	*	*	ns	ns	ns	ns	ns		
	Y×C×T	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		
		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		
		ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		

Values are the mean ± standard deviation (n=3). For a given year and cultivar, values with different letters are significantly different ($p < 0.05$, Duncan's test). For three-way ANOVA results, only $\omega^2 > 5\%$ are indicated in parenthesis; ns (not significant); * ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$)

HA1, Neochlorogenic acid; HA2, 3-p-Coumaroylquinic acid; HA3, Chlorogenic acid; HA4, 4-Caffeoylquinic acid; HA5, 4-p-Coumaroylquinic acid; HA6, di-Caffeoylquinic acid; AN1, Cyanidin 3-O-glucoside; AN2, Cyanidin 3-O-rutinoside; AN3, Peonidin 3-O-rutinoside. FA1, Procyanidin dimer B1; FA2, (+)-Catechin; FA3, Procyanidin dimer B2; FA4, (-)-Epicatechin. FO1, Quercetin triglycoside; FO2, Quercetin 3-O-rutinoside; FO3, Kaempferol 3-O-rutinoside

influenced ($p < 0.001$) the variability of quercetin triglycoside ($\omega^2 = 14\%$) and to a lesser extent ($p < 0.05$) that of kaempferol 3-O-rutinoside (FO3; $\omega^2 = 8\%$) and quercetin 3-O-rutinoside ($\omega^2 < 5\%$). The treatment factor only affected quercetin 3-O-rutinoside ($p < 0.001$, $\omega^2 = 10\%$). A significant interaction year \times cultivar was also observed for kaempferol 3-O-rutinoside ($p < 0.01$; $\omega^2 = 9\%$) and quercetin triglycoside ($p < 0.05$; $\omega^2 < 5\%$). As shown in Table 3, for both years and both cultivars, levels of quercetin triglycoside and kaempferol 3-O-rutinoside remained constant in control and HWT fruits, whether after CS and/or SL. As regards quercetin 3-O-rutinoside, despite some significant differences, no clear trend could be observed.

Conclusion

In conclusion, our results showed that the HWT conditions examined in this study (48 °C, 2 min) significantly reduced the percentage of rotten fruit after storage and/or shelf-life, without having any adverse effects, either on the level of bioactive compounds (vitamin C, polyphenols), or on the main cherry quality parameters (SSC, TA, sugars, organic acids). This study not only confirms that hot water treatment is an effective method against post-harvest rot in cherries, but also that it's a good strategy both for sweet cherry professionals and for consumers. For the former, because it's a simple method to apply to significantly reduce post-harvest losses, and for the latter, because it's a natural method that preserves the organoleptic and nutritional quality of cherries without using synthetic chemicals.

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Author contributions All authors conceived and designed the study. FB and GC performed the experiments and acquired data. CA performed statistical analysis and wrote the paper. All authors contributed to the discussion of the data and critically revised the manuscript.

Data availability The data that support the findings of this study are available on request from the corresponding author.

Declarations

Conflict of interest The authors declare that they have no conflicts of interest.

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