# Selected plant metabolites involved in oxidation-reduction processes during bud dormancy and ontogenetic development in sweet cherry buds (*Prunus avium* L.).

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**Supplementary information S1: Sugar identification by HPTLC**

The sugars (all >98 %; vwr, Darmstadt, Germany) were dissolved in water together in a stock solution mixture (1 mg.mL$^{-1}$ each). The standard solution mixture was prepared by pipetting 100 µL of the stock solution mixture together with 1.5 mL methanol in a sampler vial (63 ng·µL$^{-1}$ each). The sample (1 µL) and standard mixture solutions (1-10 µL) were applied bandwise on the HPTLC plate silica gel 60 ((20 cm × 10 cm; Merck, Darmstadt, Germany). The following settings of the Automatic TLC Sampler 4 (ATS 4, CAMAG) were used for application of 19 tracks: dosage speed 150 nL·s$^{-1}$, band length 8 mm, track distance 9.5 mm, distance from lower edge 8 mm and side edge 15 mm. After tank saturation in the Automatic Developing Chamber (ADC 2, CAMAG) for 15 min, chromatography was automatically performed with 12 mL n-butanol – i-propanol – glacial acetic acid – aqueous 2 % boric acid solution 6:14:1:3 (V/V/V/V) up to a migration distance of 60 mm according to a previous method [1]. The chromatogram was dried 1 min before and 2 min after the development. For post-chromatographic derivatization, the chromatogram was immersed in the aniline diphenylamine o-phosphoric acid reagent (mixture of 70 mL each of 2 % aniline and 2 % diphenylamine solutions, both in acetone, and 10 mL *o*-phosphoric acid, 85 %) via the TLC Immersion Device (immersion time 0 s, speed 3.5 cm·s$^{-1}$), followed by heating on the TLC Plate Heater (120 °C, 5 min). Stored in the refrigerator, the reagent was stable for weeks. The chromatograms were documented at UV 366 nm and white light illumination in transmission/reflection mode using the TLC Visualizer. Densitometry via absorption measurement at 380 nm was performed with TLC Scanner 4 and winCATS software using a slit dimension of 6.0 mm x 0.1 mm and a scanning speed of 10 mm·s$^{-1}$. Non-derivatized marked zones were directly eluted with 100 % methanol at a flow rate of 0.1 mL·min$^{-1}$ (HP 1100 ChemStation pump, Agilent, Waldbronn, Germany) via the TLC-MS Interface (CAMAG, 4 mm x 2 mm oval elution head installed) into the electrospray ionization (ESI) single quadrupol MS (ExpressIon CMS, Advion, Ithaca, NY, USA).

**Supplementary Table S1**

Determination of the different phenological development stages from leaf fall (LF) in DOY (day of the year) for the seasons 2014/15 to 2016/17 as published in [2,3]; $t_1$ = endodormancy release, $t_1$*= beginning of ontogenetic development, SB = swollen bud, SG = side green, GT = green tip, TC = tight cluster.

| **Stage** | **2014/15** | **2015/16** | **2016/17** |
|---|---|---|---|
| **LF** | 322 | 307 | 313 |
| **$t_1$** | 343 | 328 | 341 |
| **$t_1$*** | 41 | 61 | 45 |
| **SB** | 76 | 89 | 66 |
| **SG** | 86 | 92 | 76 |
| **GT** | 92 | 97 | 82 |
| **TC** | 105 | 102 | 90 |
| **OC** | 107 | 105 | 94 |

**Supplementary Table S2**

Duration (D in d) and average temperature (T in °C) observed during the different development stages for the seasons 2014/15 to 2016/17 as published in [2,3]; $t_1$ = endodormancy release, $t_1$* = beginning of ontogenetic development, SB = swollen bud, SG = side green, GT = green tip, TC = tight cluster, M = mean, SD = standard deviation.

| Stage | 2014/15 | | 2015/16 | | 2016/17 | | M ± SD | |
|---|---|---|---|---|---|---|---|---|
| | D | T | D | T | D | T | D | T |
| LF-$t_1$ | 21 | 2.6 | 21 | 9.4 | 28 | 3.0 | 23.3 ± 4.0 | 5.0 ± 3.8 |
| $t_1$-$t_1$* | 63 | 2.8 | 98 | 3.7 | 70 | 0.6 | 77.0 ± 18.5 | 2.4 ± 1.6 |
| $t_1$*-SB | 35 | 4.1 | 28 | 4.7 | 21 | 5.8 | 28.0 ± 7.0 | 4.9 ± 0.9 |
| SB-SG | 10 | 6.7 | 3 | 7.4 | 10 | 5.8 | 7.7 ± 4.0 | 6.6 ± 0.8 |
| SG-GT | 6 | 6.6 | 5 | 12.8 | 6 | 7.6 | 5.7 ± 0.6 | 9.0 ± 3.3 |
| GT-TC | 13 | 8.3 | 5 | 9.2 | 8 | 9.4 | 8.7 ± 4.0 | 9.0 ± 0.6 |
| TC-OC | 2 | 13.5 | 3 | 11.4 | 4 | 13.7 | 3.0 ± 1.0 | 12.9 ± 1.3 |

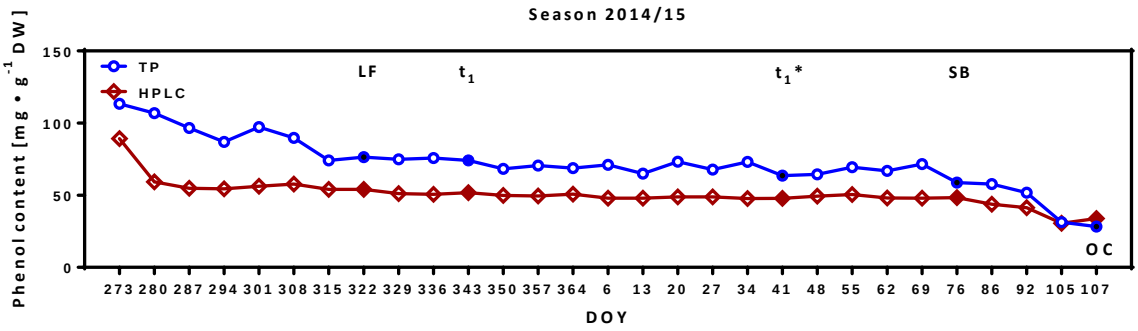


Figure S1: Comparison of the weekly and development orientated changes of the content of phenolic compounds for season 2014/15 as determined by HPLC and total phenols by Folin-Ciocalteu phenol method (TP). Filled symbols indicate the timings. Abbreviations: LF, leaf fall; $t_1$ = endodormancy release, $t_1$*= beginning of ontogenetic development, SB = swollen bud, SG = side green, GT = green tip, TC = tight cluster, OC = open cluster; DOY, day of year; DW, dry weight.

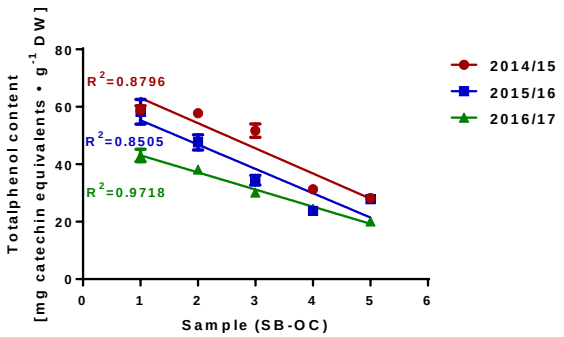


Figure S2: Correlation (p = 0.008) of the content of total phenolic compounds for the timings SB – OC of the three seasons 2014/15 - 2016/17. Slope is different for each data set (season). The correlation factors are for mean of 4 replications, error bars show the standard deviations. Abbreviations: SB (1) = swollen bud, SG (2) = side green, GT (3) = green tip, TC (4) = tight cluster, OC (5) = open cluster; DW, dry weight. Respective sample numbers are given in brackets.

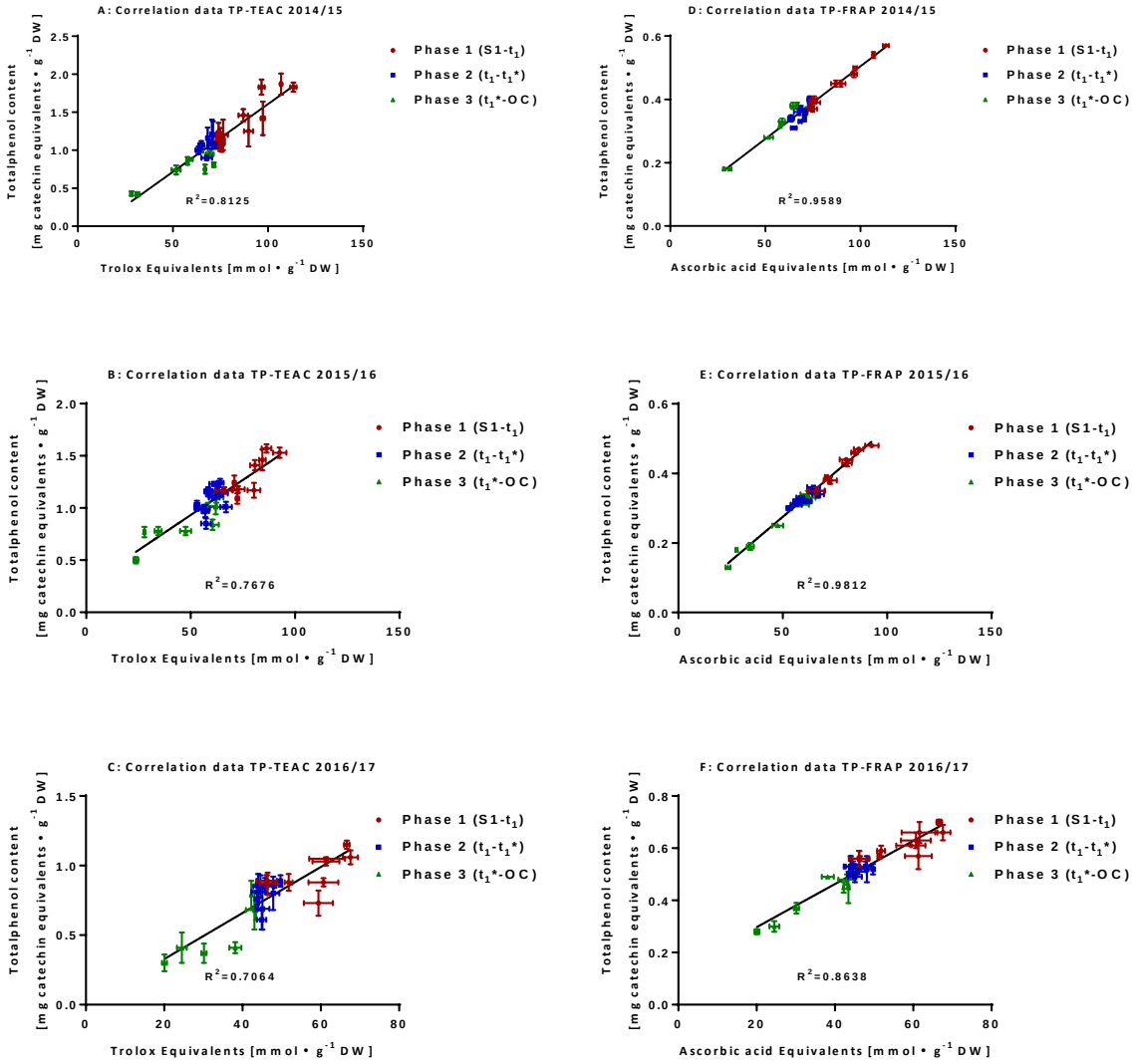


Figure S3: Correlation of the content of total phenolic compounds to the anti-oxidative potential (data presented as trolox (TEAC) or ascorbic acid (FRAP) equivalents) for the three seasons 2014/15 - 2016/17. The illustration shows the three phases of weekly and development orientated changes ($S_1$-$t_1$; $t_1$-$t_1$*; $t_1$*-OC). The correlation factors are for mean of 4 replications, error bars show the standard deviations. Abbreviations: S1 = first sampling date, $t_1$ = endodormancy release, $t_1$* = beginning of ontogenetic development, OC = open cluster, DW, dry weight.

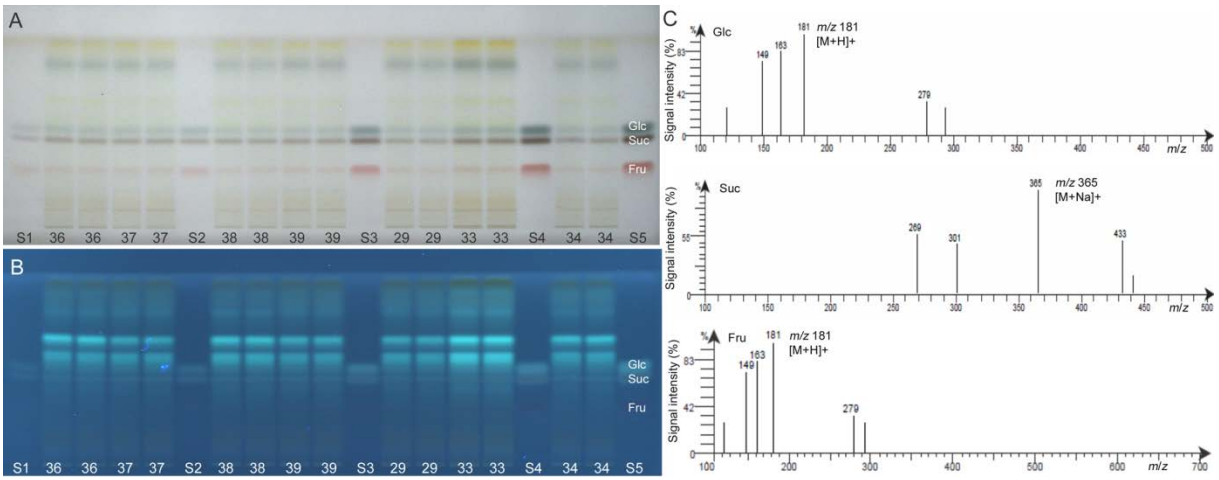


Figure S4: HPTLC separation/identification of sugars (season 2014/15) in cherry blossom bud extracts (1 µL/band; no. 29,33,34,36-39) besides standard mixture (S1-S5; 63-630 ng/band) derivatized with the aniline diphenylamine o-phosphoric acid reagent and documented at (A) white light illumination and (B) UV at 366 nm as well as (C) HPTLC-ESI$^+$-MS spectra of the three main sugars.

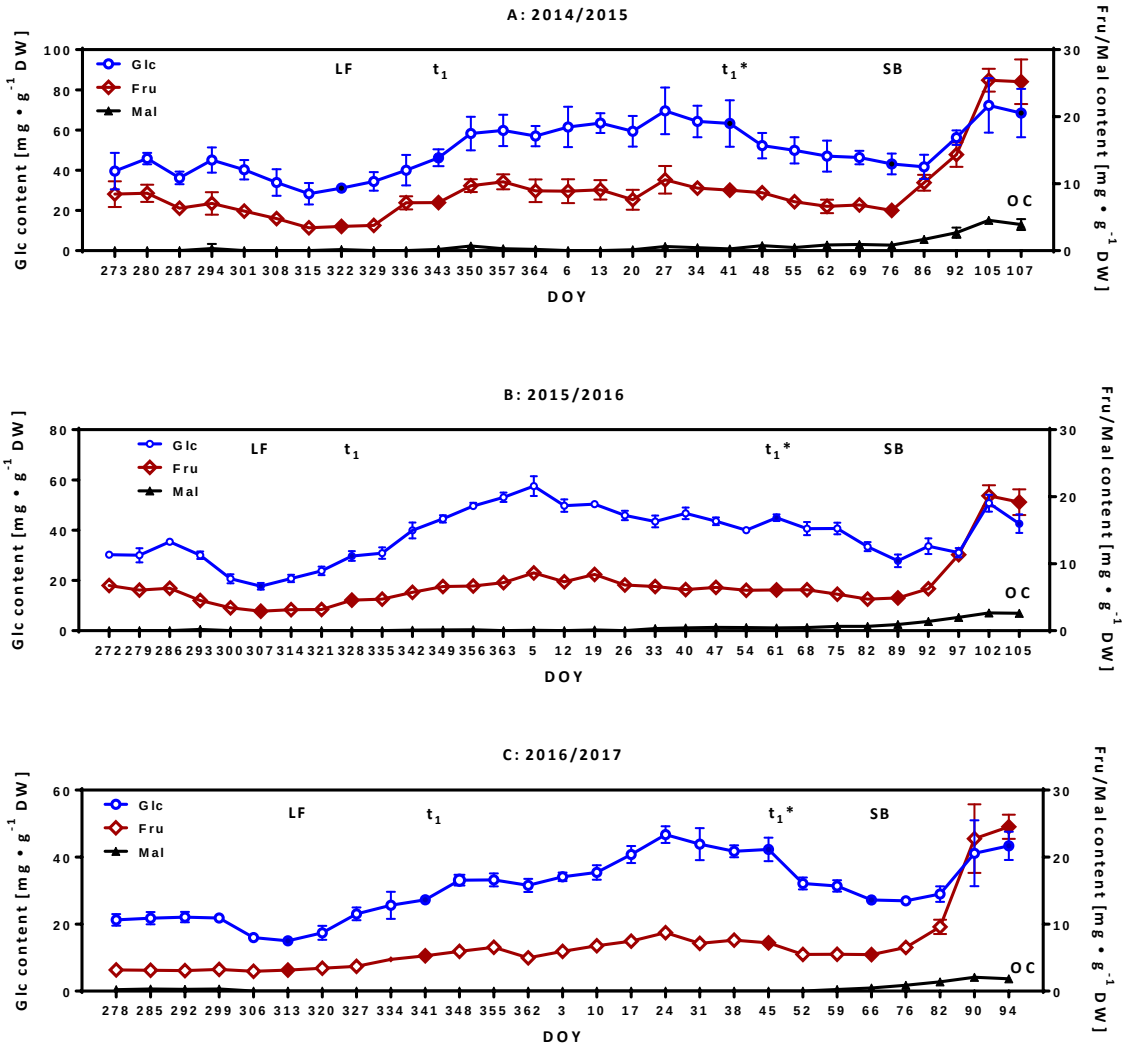


Figure S5: Weekly and development orientated changes of the content of reducing sugars. A: Season 2014/15; B: Season 2015/16 and C: Season 2016/17. Filled symbols indicate the timings. Abbreviations: Glc, glucose; Fru, fructose; Mal, maltose; LF, leaf fall; $t_1$ = endodormancy release, $t_1$*= beginning of ontogenetic development, SB = swollen bud, SG = side green, GT = green tip, TC = tight cluster, OC = open cluster, DOY, day of year; DW, dry weight.

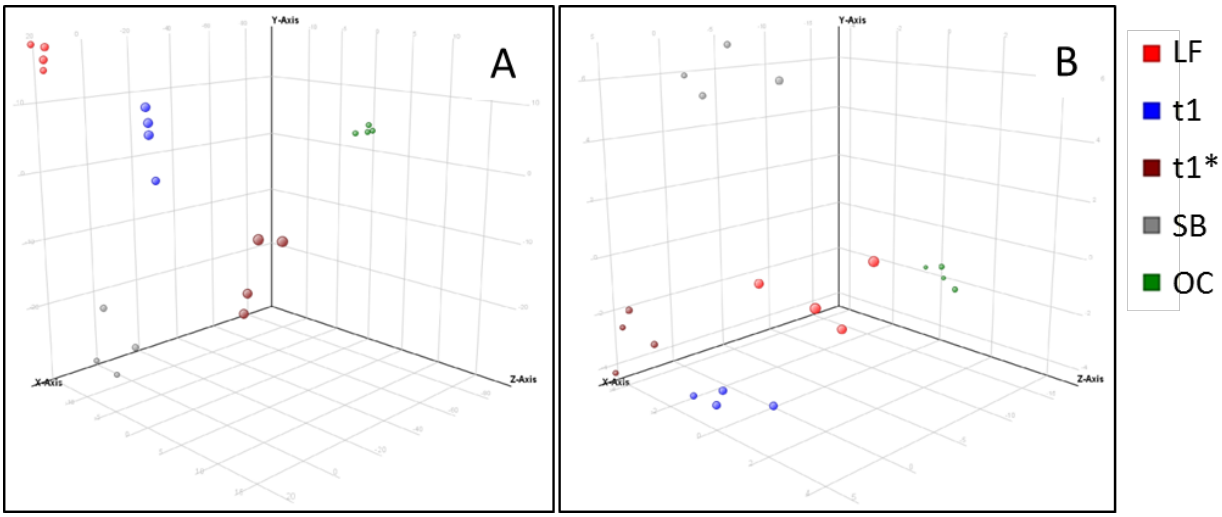


Figure S6: Principal component analysis ($p \leq 0.05$) on the metabolites present during the different timings of the development stages as determined by untargeted MS analysis for the season 2014/15; *m/z*: 100-1700. A: Fold change ≥ 2 in negative modus with PC1= 66.06% (x-axis), PC2=7.79% (y-axis) and PC3=3.72% (z-axis). B: Fold change ≥ 1.5 in positive modus with PC1= 60.8% (x-axis), PC2=9.99% (y-axis) and PC3=6.27% (z-axis). Abbreviations: LF, leaf fall; $t_1$ = endodormancy release, $t_1$*= beginning of ontogenetic development, SB = swollen bud, OC = open cluster.

Supplementary Table S3: Log fold change (up- and down regulation) of selected metabolites as compared to LF present during the different timings of the development stages as determined by untargeted MS analysis for the season 2014/15. Abbreviations: LF, leaf fall; $t_1$ = endodormancy release, $t_1$*= beginning of ontogenetic development, SB = swollen bud, OC = open cluster; n.d. = not detected.

| **Compound/ Stage** | **$t_1$** | **$t_1$*** | **SB** | **OC** |
|---|---|---|---|---|
| Glutathione, oxidzed | n.d. | -4.09 | -8.27 | -16.09 |
| Glutathione, reduced | 4.80 | 19.11 | 18.25 | 15.44 |
| Asp Met Trp | 17.58 | 17.98 | 17.97 | 18.66 |
| Maltotetraose | 19.41 | -13.73 | -18.18 | -18.18 |
| 4'-Hydroxyacetophenone | -16.96 | n.d. | -4.47 | -16.96 |
| Epigallocatechin | 5.24 | 5.38 | -9.22 | -13.75 |
| Methyl 3,4-dicaffeoylquinate | 14.43 | 4.70 | -4.71 | -4.71 |
| Phosphatidyl glycerol | -9.08 | -9.00 | -17.52 | n.d. |
| Phytosphingosine | -4.28 | 8.42 | -8.39 | -8.39 |
| Dehydrophytosphingosine | -9.76 | -13.07 | -1.58 | 2.40 |

**References**


1. Morlock, G.E.; Sabir, G. Comparison of two orthogonal liquid chromatographic methods for quantitation of sugars in food. *J. Liq. Chromatogr. Relat. Technol.* **2011**, *34*, 902-919.
2. Chmielewski, F.M.; Baldermann, S.; Gotz, K.P.; Homann, T.; Schumacher, F.; Huschek, G.; Rawel, H. Abscisic acid related metabolites in sweet cherry buds *J. Hort.* **2018**, *5*, 221.
3. Chmielewski, F.M.; Gotz, K.P. Identification and timing of dormant and ontogenetic phase for sweet cherries in northeast Germany for modelling purposes. *J. Hort.* **2017**, *4*, 205.