Abstract: Chilean fresh blueberries take 20-50 days to arrive by boat to the Northern hemisphere, softening and dehydration being the main defects upon arrival. The effect of maturity at harvest (75% blue, 100% blue, and overripe) on cuticular triterpene content, and the possible associated impacts on firmness and weight loss after cold storage were explored for 'Duke' and 'Brigitta' fruit, both non-bagged or bagged in macro-perforated low-density polyethylene bags. Softening and weight loss varied with cultivar and maturity stage: 'Duke' fruit softened faster and were more prone to dehydration than 'Brigitta' samples, whereas overripe fruit were less firm after storage. This is the first report characterizing the triterpenoid fraction in cuticles of fresh blueberries, which may play a role in their postharvest behavior. Weight loss and softening rates were highly correlated to ursolic acid contents at harvest; further research will be required for a better understanding of these relationships.
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Please accept the submission of the following manuscript entitled “Fruit Characteristics and Cuticle Triterpenes as Related to Postharvest Quality of Highbush Blueberries” for review and eventual publication in SCIENTIA HORTICULTURAE. We hope you will consider the content relevant for the scope of the Journal, and the manuscript suitable for publication. Our target was to explore the effect of fruit maturity stage and cuticle triterpene content at harvest on postharvest fruit quality of two relevant blueberry cultivars. To our knowledge, this is the first report that characterizes cuticular triterpenoid composition of fresh blueberries, and its relationship with postharvest fruit quality.

This manuscript is an original contribution, and it is not being under consideration for publication, published or accepted for publication in any other journal or book. Its submission for publication has been approved by all relevant authors and institutions, and all persons entitled to authorship have been so named. We look forward to the eventual appearance of this work in Scientia Horticulturae. Should you have any questions regarding the manuscript, please do not hesitate to contact me at cmoggia@usalca.cl as the corresponding author.

Sincerely yours,

Claudia Moggia
Universidad de Talca, Chile
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Graphical Abstract

Blueberry maturity stages

Graph 1: Percentage of Triterpenes (Amyrin, Lupeol, Oleanolic Acid, Ursolic Acid) in Duke and Brigitta varieties.

Graph 2: Relationship between Ursolic acid content (g/m²) and % Weight loss (r = 0.96**).

Graph 3: Relationship between Ursolic acid content (g/m²) and % Firmness loss (r = 0.95**).
First report characterizing blueberry fruit cuticular triterpenes

Ursolic acid was positively correlated to weight loss and softening after storage

Lupeol was the main triterpene, while α-amyrin was only present in Briggita

Duke, firmer at harvest, was highly affected by harvest delay compared to Brigitta
Fruit Characteristics and Cuticle Triterpenes as Related to Postharvest Quality of Highbush Blueberries

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Abstract

Chilean fresh blueberries take 20-50 days to arrive by boat to the Northern hemisphere, softening and dehydration being the main defects upon arrival. The effect of maturity at harvest (75% blue, 100% blue, and overripe) on cuticular triterpene content, and the possible associated impacts on firmness and weight loss after cold storage were explored for ‘Duke’ and ‘Brigitta’ fruit, both non-bagged or bagged in macro-perforated low-density polyethylene bags. Softening and weight loss varied with cultivar and maturity stage: ‘Duke’ fruit softened faster and were more prone to dehydration than ‘Brigitta’ samples, whereas overripe fruit were less firm after storage. This is the first report characterizing the triterpenoid fraction in cuticles of fresh blueberries, which may play a role in their postharvest behavior. Weight loss and softening rates were highly correlated to ursolic acid contents at harvest; further research will be required for a better understanding of these relationships.

Key words: blueberry; cuticle; firmness; fruit; triterpenoids; *Vaccinium corymbosum* L.; weight loss
1. Introduction

Chile has a large fresh blueberry-exporting industry (Retamales and Hancock, 2012) and, owing to counter-seasonality, it has the commercial advantage of supplying off-season fresh fruit to the Northern hemisphere. In order to reduce shipping costs, transportation by boat is the preferred means of export (Beaudry et al., 1998). Currently, the proportion of fruit shipped by boat is around 95%, and transport may take 20 to 50 days, from harvest to final consumers. The main market for Chilean fresh blueberries is the USA (82 - 85% of the total volume exported in 2008 - 2011), followed by Europe (12 - 14%) and the Far East (3%), (ODEPA, 2015). Fresh blueberries are relatively perishable, so considering the actual extreme variations in weather patterns due to the climate change (Lobos and Hancock, 2015) and the increasing amount of fruit shipped to long-distance markets, quality upon arrival is likely to become more heterogeneous and this will become a major issue for the blueberry industry (Retamales et al., 2014).

Blueberries are prone to postharvest decay, physiological breakdown, physical damage, shriviling, and water loss. The quality at final markets is dependent on the attributes of fruit at harvest, as well as on handling during and after harvest (Forney, 2009). Fruit softening is one of the major factors limiting the marketing of fresh blueberries (Vicente et al., 2007) and also one of the most critical quality attributes that influence consumer acceptance (NeSmith et al., 2002). According to the industry, the main defects found in Chilean blueberries at final markets are fruit softening and dehydration, accounting for 10 - 45% and 10 - 25% of total defects, respectively (Juillerat, 2014).

In general, fruit softening is estimated by the instrumental measurement of firmness, which declines with maturation. Firmness can vary greatly among cultivars, but also across maturity stages within a singular cultivar (Beaudry, 1992; Lobos et al., 2014). Additionally, blueberries
usually soften during the postharvest chain due to deficient temperature management (Ehlenfeldt and Martin, 2002; Tetteh et al., 2004; Ne Smith et al., 2015), although a number of studies have also reported increases in firmness during storage (Miller et al., 1993; Chiabrando et al., 2009; Duarte et al., 2009). Research on blueberry fruit softening has focused on metabolic changes in the cell walls, leading to structural disassembly, which appears to be almost completed by the time of harvest (Vicente et al., 2007; Angeletti et al., 2010), while other possibly involved factors have not been deeply studied. The fruit cuticle, for instance, has a noticeable influence on the postharvest quality of fruits, on three major aspects: water permeability with the resulting dehydration, susceptibility to infections, and physiological disorders (Lara et al., 2014).

The cuticle is a mostly lipidic external membrane surrounding all non-woody aerial plant organs (Dominguez et al., 2011). Its main component is cutin, a polyester matrix of polyhydroxylated \( \text{C}_{16} \) and \( \text{C}_{18} \) fatty acids embedded and covered with amorphous intra- and epicuticular waxes, plus a minor fraction of phenolics (Jetter et al., 2000). Cuticular waxes are composed of mixtures of aliphatic (\( n \)-alkanes, alkanoic acids, alkanols, aldehydes, alkyl esters), and non-aliphatic components (pentacyclic triterpenoids and sterol derivatives) (Kunst and Samuels, 2009). Recent studies on tomato (Lleide et al., 2011), pepper (Parsons et al., 2012), sweet cherry (Belge et al., 2014a), and peach (Belge et al., 2014b) have demonstrated a positive association between water loss rate and the ratio of \( n \)-alkanes to triterpenoids plus sterol compounds. For the edible berries within the genus *Vaccinium*, most available information refers to cranberry (*Vaccinium macrocarpon*), which is known to be a rich source of the triterpenoids ursolic and oleanolic acids (Crouteau and Fagerson, 1971; Szakiel et al., 2012), whereas Kondo et al. (2010) detected the same compounds in lowbush blueberries (*Vaccinium angustifolium*). We
are not aware, though, of any reports on the specific composition of *Vaccinium corymbosum* fruit cuticles.

Interestingly, moisture loss has been recently proposed as the major cause of firmness changes during storage of blueberries (Paniagua et al., 2013). There is evidence that cuticle characteristics and composition might play a role on softening of fruits such as pepper and tomato (Bargel and Neinhuis, 2004; Maaleku et al., 2005; Kosma et al., 2010). Noticeable differences have been reported across blueberry cultivars regarding softening rates and water loss during prolonged refrigerated storage (Vicente et al., 2007; Alsmairat et al., 2011; Sargent et al., 2006; Paniagua et al., 2013; Paniagua et al., 2014), but to our knowledge, no published study has evaluated the influence of harvest maturity and cuticular wax characteristics on quality parameters during cold storage or transport.

We hypothesize that the triterpenoid content of the highbush blueberry cuticle may impact weight loss and softening of the fruit after storage. The work reported herein is a preliminary study undertaken with the main goal of assessing the relationships, if any, between quality parameters and the cuticular triterpenoids in two highbush blueberry cultivars (‘Duke’ and ‘Brigitta’) harvested at different maturity stages. Fruit were maintained under refrigerated storage, either unpacked or packed within a low-density macro-perforated polyethylene bag, to mimic shipping to long-distance markets.

2. **Material and Methods**

2.1. *Fruit material and experimental setup*

During the season 2014/15, twelve mature highbush blueberry (*Vaccinium corymbosum* L.) plants of ‘Duke’ and ‘Brigitta’, 8 and 9 years old, respectively, planted 1.2 m apart in rows
spaced at 3 m, were selected and labeled from a commercial field located in Río Claro, Maule Region, Chile (35°15'35.16" S; 71°14'22.53" W). Early in the season, when similar percentages of green and pink fruit were reached, clusters with comparable characteristics (fruit number and shape) and canopy position (superior third of the eastern side), were selected and labeled. Fruit ripeness was categorized according to external color as: 75% blue color and pink button (H75), 100% blue and residing on the plant for a maximum of 2 days (H100), and 100% blue and residing on the plant for 5 to 7 days (H100+X). No visual differences in the skin color could be perceived between H100 and H100+X fruit. The latter maturity stage was imposed to mimic the usual commercial harvest practice. The extent of ripening was evaluated every second day, in order to get the different maturity stages. There were three harvest dates for each cultivar: 26 and 29 November, and 5 December (H75, H100 and H100+X, correspondingly) for ‘Duke’; 27 and 31 December, and 5 January for ‘Brigitta’ (H75, H100 and H100+X, respectively).

Fruit from each maturity stage and cultivar were carefully hand-picked and placed directly into plastic clamshells (125 g), containing 50 fruit each. In order to mimic real conditions, fruit were placed in commercial cardboard boxes (containing 12 clamshells), for each cultivar and maturity. Fruit from four clamshells were evaluated at harvest, whereas the remaining fruit were divided into two storage treatments: i) four boxes were placed within a commercial macro-perforated (0.9%), low-density polyethylene (LDPE) unsealed bag, which was used only for weight loss prevention and no gas modification was intended (Pesis et al., 2002; Klaasen et al., 2006; Koutsimanis et al., 2015); and ii) four boxes remained non-bagged as the control. Fruit were stored at 0 °C and evaluated after 45 days at 0 °C plus 1 day at 18 °C (45+1). The general experiment was established under a completely randomized design, with factorial arrangement given by maturity stage (3) and bagging system (2), thus generating three treatments at harvest.
and six treatment combinations for the postharvest evaluations. Each treatment had four replicates (one clamshell e.a.).

2.2. Maturity and quality assessments

Fruit weight (g) was measured with an electronic balance, and equatorial and polar diameters (mm) were measured with a digital caliper on four replicates of 25 fruit each. On the same lot, firmness (N) was measured with a compression device (FirmTech 2, BioWorks, KS, USA); the equipment was set up with maximum and minimum compression forces of 1.96 N and 0.15 N, respectively, and piston speed of 6 mm s⁻¹ (Ehlenfeldt and Martin, 2002; Saftner et al., 2008).

Total soluble solids (TSS, %) were assessed in juice obtained from four replicates of 5 berries each with a digital refractometer (Pocket PAL-1, Atago, Tokyo, Japan). For the determination of titratable acidity (TA, % citric acid), four replicates of 10 mL of juice were diluted to 100 mL with distilled water and titrated with 0.1 mol L⁻¹ NaOH to an end-point pH of 8.2. Additionally, the ratio between TSS/TA was calculated. For the evaluation of respiration rate (RR), samples (three fruit × four replicates) were placed within 28-mL sealed glass vials. After 2 h at room temperature (18 °C), CO₂ accumulation inside the vials was measured using a gas analyzer (Quantek 902P, Quantek Instruments Inc., MA, USA) fitted with a thermal conductivity detector; CO₂ production was expressed as μg kg⁻¹ s⁻¹. An authenticated standard (2.1 % CO₂ and 2.2 % O₂ in N₂ balance) was used for calibration. Additional samples were also placed in 28-mL vials for the measurement of ethylene production (EP); after 2 h at room temperature (18 °C), a 1 mL gas sample was withdrawn with a syringe from the headspace volume, and ethylene was quantified using a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) equipped with a flame ionization detector and a 3 mm i.d. column packed with activated alumina, 80/100 mesh. The
injector, oven, and detector temperatures were set at 75 °C, 100 °C, and 170 °C, respectively, with helium as the carrier gas (0.67 mL s\(^{-1}\)), in the presence of hydrogen and air (0.67 and 6.67 mL s\(^{-1}\), correspondingly). An ethylene standard (1 \(\mu\)L L\(^{-1}\)) was used for calibration, and data were expressed as ng kg\(^{-1}\) s\(^{-1}\). For RR and EP, the free headspace of each vial was estimated by subtracting the fruit volume from the total volume of each vial. Fruit volume and surface were calculated using the polar and equatorial diameters of each berry, assuming an oblate spheroid shape. Additionally, surface/volume ratios were estimated for each maturity stage.

After storage removal (45+1), firmness, TSS and TA were measured for both bagged and non-bagged fruit. Firmness was assessed on four replicates of 25 fruit each; TSS and TA were measured on four replicates of 5 berries and four replicates of 10 mL juice, respectively. Weight loss (%) was estimated by the difference between initial and final weight on four replicates of one clamshell, per treatment. Given that fruit size differed between cultivars, weight loss was also expressed as % m\(^{-2}\).

Finally replicates of 50 fruit were visually evaluated to determine the % of sound fruit (edible berries, free of any shriveling and/or rot symptoms) on each clamshell.

2.3. Fruit cuticular wax analysis and triterpenoid identification/quantification

Cuticular wax analyses were undertaken on fruit at harvest. In order to avoid wax removal during picking of fruit used for wax analysis, entire clusters were collected at the field, set into paper bags, and once at the lab, individual fruit were removed from the clusters with tweezers, holding each berry from the pedicel. Fruit wax was extracted (three replicates of 25 fruits e.a.) by dipping the samples in 50 mL distilled dichloromethane, with continuous agitation for 1 min. The solution was filtered and taken to dryness under reduced pressure at 30 °C in a
rotatory evaporator. The solid residue obtained from each replicate sample was dried and weighed to estimate wax yield, per unit surface area (g m\(^{-2}\)). The composition of the wax extracts was first assessed by thin layer chromatography (TLC) analysis (silica gel 60 F254, Merck, Darmstadt, Germany) using petroleum ether:ethyl acetate 90:10 (v/v) as the mobile phase. Plates were visualized after spraying with anisaldehyde-sulfuric acid and heating. Several spots were detected with colors suggesting the occurrence of triterpenes and triterpene acids. Selected samples were treated with diazomethane in diethyl ether to obtain the methyl esters of triterpene acids. For triterpenoid identification and quantification by GC-MS, the samples (1 g L\(^{-1}\) of wax extract) were treated with 1 mL of diazomethane solution in diethyl ether to obtain the methyl esters of the acids occurring in the mixtures. After evaporation to dryness, the derivatized samples were dissolved in isopropanol and analyzed by GC-MS. The presence of a mixture of triterpene alcohols and triterpene acids was confirmed by \(^1\)H NMR analysis (400 MHz, Bruker, Rheinstetten, Germany). The main triterpenes in the samples were identified by analysis of the lipophilic cuticle constituents by TLC, GC-MS and NMR before and after derivatization as the corresponding methyl esters. The identity of the compounds was confirmed by comparison with authentic standards of oleanolic acid, ursolic acid, lupeol and \(\alpha\)-amyrin.

Further analysis and quantification were carried out by GC. For quantification, cholesterol (Sigma-Aldrich C 8667, purity \(\geq 99\%\)) was used as internal standard.

2.3.1. Chemical Standards and Reagents

Dichloromethane, ethyl acetate, petroleum ether and diethylether were from Merck (Darmstadt, Germany). Isopropanol was from J.T. Baker (Center Valley, PA, USA). Oleanolic acid (O5504, purity \(\geq 97\%\)), ursolic acid (89797, purity \(\geq 98.5\%\)), \(\alpha\)-amyrin (53017, purity \(\geq\) ...
191 98%) and lupeol (L5632, purity ≥ 94%) were from Sigma-Aldrich (St. Louis, MO, USA).
192 Cholesterol (Sigma-Aldrich C8667, purity ≥ 99%) was used as internal standard.
193
194 2.3.2. Identification
195
196 The identification of the compounds was carried out using a gas chromatograph (GC Trace
197 1300, Thermo Fisher Scientific, Milan, Italy) coupled to a mass selective detector fitted with an
198 ionization single quadrupole according to Caligiani et al. (2013). A capillary column (0.25 mm
199 i.d., 30 m length × 0.25 μm film thickness) was used (Rtx-5, Restek Corporation, PA, USA). The
200 oven temperature was kept at 240 °C for 3 min then increased to 280 °C at 20 °C min⁻¹, with a
201 total running time of 60 min. The head pressure was 124 kPa. Both the injector and detector
202 temperatures were 290 °C, with 0.2 min split-less injection mode. One μL was injected, with
203 helium as the carrier gas at 25 μL s⁻¹. For mass spectrometric (MS) analyses, the ion source
204 temperature was 230 °C (70 eV, m/z 50−700). Under the experiment conditions, the retention
205 time (Rt) of the internal standard and triterpenes were as follows: cholesterol (12 min), α-amyrin
206 (17 min), lupeol (18 min), oleanolic acid methyl ester (23 min) and ursolic acid methyl ester (25
207 min).

208 2.3.3. Quantification
209
210 Compounds were quantified with a gas chromatograph (GC Trace 1300, Thermo Fisher
211 Scientific, Milan, Italy), coupled to an FID. A capillary column (0.25 mm i.d., 30 m length ×
212 0.25 μm film thickness) (Elite-5MS, PerkinElmer, MA, USA) was used. The oven temperature
213 was held at 240 °C for 3 min, and then increased to 280 °C at 20 °C min⁻¹, with a total run time
214 of 45 min. Helium was used as the carrier gas (25 μL s⁻¹). The injected volume was 1 μL in all
cases, with both injector and detector maintained at 290 °C, and operated for 0.2 min in a
splitless injection mode. Air (5.83 mL s\(^{-1}\)) and hydrogen (0.58 mL s\(^{-1}\)) were used as the carrier
gas. The quantification was done by integrating the total area of each chromatographic peak with
cholesterol as internal standard at a concentration of 1 g L\(^{-1}\). Results were expressed in mg m\(^{-2}\) as
well as in relative terms (% of each compound over total waxes).

2.4. Statistical analysis

Data were subjected to analyses of variance (ANOVA). The significance of the differences
was determined by Tukey’s test (\(p \leq 0.05\)). In order to aid a preliminary characterization of the
influence of the factors considered (cultivar, maturity stage, cuticle triterpenoid composition and
bagging) on fruit characteristics, regression analyses were performed to relate weight loss with
fruit maturity and characteristics at harvest. Analyses were executed using commercial statistical
software (Statgraphics Centurion XVI (v.16.0.09), Statpoint, VA, USA) and R 3.0.0 (R
Development Core Team, 2008).

3. Results

3.1. Fruit maturity and quality assessments at harvest

For H75 and H100, fruit firmness was similar, but higher than at H100+X for both cultivars
(Table 1). ‘Duke’ showed significant differences among the three stages for TSS and TSS/TA,
whereas for ‘Brigitta’ there were no differences between H100 and H100+X for TSS, TA, or
TSS/TA. Regarding EP, values were below 0.5 ng kg\(^{-1}\) s\(^{-1}\), with no differences between maturity
stages for either cultivar. For ‘Duke’, H75 and H100+X fruit had higher RR than H100 fruit,
whereas RR values for ‘Brigitta’ were lower than those for ‘Duke’, and decreased as maturity
increased from H75 to H100+X (Table 1).

Maximum fruit weight was reached at H100 in ‘Duke’ and H100+X in ‘Brigitta’ (Table 2).

In terms of fruit size, both cultivars grew equatorially until the fruit lost any trace of pink color (H100); polar diameter increased until H100+X in ‘Duke’, whereas ‘Brigitta’ did not show differences between stages. Surface/volume ratios were higher for ‘Duke’ blueberries and decreased from H75 to H100 in both cultivars. No differences in total wax content were found among maturity stages for either cultivar, even though contents were slightly higher in ‘Duke’ (Table 2).

3.2. Fruit cuticle triterpenoids at harvest

Two triterpenoid alcohols (α-amyrin and lupeol), as well as two triterpenoid acids (oleanolic and ursolic acids), were identified in the triterpenoid fraction of total waxes from ‘Duke’ and ‘Brigitta’ blueberries by spectroscopic and spectrometric means. GC traces of the wax constituents are presented as Supplementary Figures S1 and S2. There were no differences in the total % of triterpenoid components between maturity stages for ‘Duke’ (49% on average), but some dissimilarities were apparent for ‘Brigitta’, for which the content of triterpenoids was 45% for H75 and H100, and around 35% for H100+X (Table 3).

The main compound identified in both cultivars was lupeol (Fig. 1), which was more abundant in ‘Duke’, where it increased with maturity stage from 1.16 to 2.03 g m$^{-2}$. Lower values of this triterpene were found in ‘Brigitta’ (0.35 to 1.41 g m$^{-2}$), increasing from H75 to H100, and decreasing towards H100+X.

Large differences between cultivars were also found for oleanolic and ursolic acids. The content of oleanolic acid averaged 0.37 g m$^{-2}$ in ‘Brigitta’, with no maturity-related differences,
the amounts being about two-fold those in ‘Duke’. In contrast, ‘Duke’ waxes were 2- to 7-fold higher in ursolic acid content in comparison with levels in ‘Brigitta’, although the amounts decreased with maturity. Finally, the triterpene alcohol α-amyrin was detected in ‘Brigitta’ fruit uniquely, and amounted on average to 0.24 g m⁻², regardless of maturity stage.

3.3. Fruit quality and weight loss after storage

After storage (45+1), firmness was influenced by the factors under study, decreasing in non-bagged fruit with advanced harvest maturity (Table 4). In general, firmness of ‘Duke’ fruit declined 32, 25 and 18% at H75, H100, and H100+X stages, respectively, in comparison with levels at harvest. For ‘Brigitta’, these decreases were 4, near 0 and 19.8%, respectively. For both cultivars, though, H75 and H100 fruit remained firmer than H100+X fruit. The impact of the bagging procedure on firmness preservation was also dissimilar between cultivars: a difference of 34 and 11.5% in firmness loss after storage was observed for ‘Duke’ and ‘Brigitta’ fruit, respectively, when comparing non-bagged and bagged samples.

TSS, TA and TSS/TA after storage were significantly affected by harvest maturity, ‘Duke’ berries showing differences among all three stages for TA and TSS/TA, while H100 and H100+X ‘Brigitta’ fruit were generally similar. For both cultivars, the TSS/TA increased after storage due to increased TSS and decreased TA. The highest values recorded for TSS/TA ratios in 45+1 fruit were 34.2 for H100+X ‘Duke’ berries, and 24.9 and 27.8 for H100 and H100+X ‘Brigitta’ samples, respectively (Table 4).

In terms of maturity, for both cultivars the percentage of sound fruit was similar for H75 and H100 samples, which were higher in comparison with H100+X fruit (Table 4); on average, 60 and 90% of the H75 and H100 berries were considered sound for ‘Duke’ and ‘Brigitta’,
respectively, but only 43 and 80% of the H100+X fruit of ‘Duke’ and ‘Brigitta’ were still sound after storage. The effect of bagging on the percentage of visually sound fruit was significant for both cultivars, but differences were larger in ‘Duke’, where 81.8% of berries were considered healthy under bagged conditions, but only 25.4% resulted free of defects when no bag was used. For ‘Brigitta’ sound fruit represented 92.2 and 81.4% of bagged and non-bagged treatment, respectively.

The effects of maturity stage and bagging on weight loss showed almost the same statistical significance for values expressed either as % or as % m^-2. Large differences were found between cultivars, ‘Duke’ being more prone to dehydration than ‘Brigitta’ in all cases (Table 4); ‘Duke’ had the highest weight loss, particularly for H75 and H100 fruit (14.4 and 12.4%; 5.8 and 5.0% m^-2, respectively), whereas values were much lower for ‘Brigitta’, ranging from 5.0 to 7.2% and 2.1 to 2.6 % m^-2. Additionally, the effect of bagging on weight loss was higher for ‘Duke’, where fruit with no bag lost 3 and 2.3 times more weight (as % and % m^-2, respectively) than bagged fruit. For ‘Brigitta’ differences between bagged and non-bagged fruit were less than 2 times.

Regression analyses for weight loss revealed significant associations (p ≤ 0.05) between fruit characteristics and wax compounds at harvest (Table 5). Thus, weight loss values (both as % and % m^-2) were highly correlated with surface/volume ratio (r = 0.91 and 0.89), EP (r = 0.94 and 0.92), ursolic acid content (r = 0.96 and 0.95) and initial fruit weight (r = -0.82 for % weight loss uniquely). Additionally, when fruit softening (expressed as % drop between initial and final firmness) was added as the response variable, significant correlations were found against fruit weight (r = -0.96), EP (r = 0.94), RR (r = 0.81), oleanolic and ursolic acid contents (r = -0.83 and 0.95, respectively).
4. Discussion

4.1. Fruit quality vs. weight loss, firmness and softening after storage

The criteria for determining harvest maturity of fresh blueberries rely mainly on surface color, which has to be 100% blue (Gough, 1994; Lobos et al., 2014). Yet, firmness and TSS/TA, which are seldom measured under commercial management, have also been associated to postharvest potential, especially for long-term storage and transport. Firm fruit can more readily withstand harvest handling and subsequent transport (Hanson et al., 1993) and even though some cultivars are only slightly firmer, such small differences can prove very important for postharvest life (Beaudry et al., 1998). Several authors have reported differences in firmness of highbush blueberry cultivars (Ehlenfeldt and Martin, 2002; Saftner et al., 2008), which however seem to be more related to harvest maturity than to genotypic differences (Beaudry et al., 1998; Lobos et al., 2014). In this study, we found that firmness after storage was related to both maturity stage (H75 and H100 fruit remained firmer than H100+X ones) and cultivar (‘Duke’ displaying higher firmness values at harvest, but faster softening rates than ‘Brigitta’ after storage). The fact that no visual differences in color could be detected at harvest between H100 and H100+X samples suggests that a relatively wide variation in maturity may exist in any one harvest. In a typical commercial harvest, fruit can be collected every 6 - 10 days, which would practically assure a wide range in fruit maturity. The consistently higher firmness of H100 relative to H100+X samples, both at harvest and after storage, illustrates the problems associated with the presence of fruit with advanced maturity in harvested fruit lots. Similarly, the TSS/TA ratio, which should be balanced in order to achieve optimal flavor, would also be impacted by variation in fruit maturity. Galletta et al. (1971) proposed that good keeping quality could be expected when TSS/TA ratios are < 18, and intermediate keeping quality when values are in the range 18-32. In
our study, H75 fruit had the lowest ratios (around 12); H100 fruit were close to the optimal threshold (roughly 20), but H100+X samples displayed TSS/TA > 24, which appear too high if long-distance markets are to be reached with acceptable quality. In terms of firmness, although no optimum parameters have been defined, mean values for ‘Duke’ at harvest have been reported between 1.73 and 1.36 N (Ehlenfeldt and Martin, 2002; Saftner et al., 2008) and for ‘Brigitta’ between 1.88 and 1.46 N (Ehlenfeldt and Martin, 2002). In our study, firmness of ‘Duke’ fruit was within the mentioned range for all the maturity stages (1.76 N for H75 fruit to 1.38 for H100+X fruit), whereas ‘Brigitta’ berries were slightly softer (1.63 vs. 1.31 N from H75 to H100+X stages).

Since growers often wait for blue fruit to accumulate in the bushes in order to optimize labor costs, it is most likely that, within each harvest, there is a relatively wide range in fruit maturity amidst the uniformly colored fruit harvested. All the fruit may look acceptable when picked, but a fraction of them, the ones picked at more advanced maturity, have a greater likelihood of becoming overripe and unacceptable when reaching the final consumers. This may be an important source of fruit heterogeneity, which will be more deleterious after longer storage and transport periods, and could partially explain quality variations detected at final markets between different seasons (Juillerat, 2014). Results for final firmness and % sound fruit after storage showed that, in terms of maturity stage, H75 and H100 stages of both cultivars, as well as H100+X of ‘Brigitta’, had a similar behavior but highly differed from those of ‘Duke’ harvested at H100+X.

Visually, and regardless of cultivar, H75 berries achieved complete blue coverage after storage, but had lower TSS and higher TA than H100 or H100+X fruit. This might have had implications for organoleptic characteristics that were not explored in this study.
'Duke' fruit were firmer at harvest and had slightly higher amount of waxes, but displayed similar TA and TSS/TA values as ‘Brigitta’. Yet, these attributes did not result in better condition after storage, since the proportion of sound fruit was substantially lower for ‘Duke’ (< 60%, depending on maturity stage) than for ‘Brigitta’ (> 80% at all stages considered herein).

Values for weight loss (expressed both as % and % m⁻²) were high and varied between both cultivars. The blueberry industry considers acceptable a range of 5 - 7% weight loss in a commercial 3-week maritime transport where fruit are containerized at 0 °C and held under 90 - 95% RH (Sargent et al., 2006; Paniagua et al., 2014). These values would be consistent with those obtained in this study for ‘Brigitta’, but not for ‘Duke’ fruit, for which a higher weight loss was observed, particularly for non-bagged fruit. When Alsmairat et al. (2011) evaluated 9 cultivars under different controlled atmosphere storage conditions, weight loss was in the range of 0.6 to 2.3% after eight weeks; among cultivars, ‘Duke’ showed two-fold higher weight loss compared to ‘Brigitta’. Rivera et al. (2013) reported 2.1 and 3.5% weight loss for palletized ‘Brigitta’ and ‘O’Neal’ blueberries, respectively, after 45 d at 0 ºC. In a recent experiment, the use of passive modified atmosphere packaging (MAP) for the storage of ‘Brigitta’ fruit resulted in decreased percentage of dehydrated fruit and less intense softening when compared to control fruit (Moggia et al., 2014) and interestingly film type had little effect on gas composition within the bag, showing that moisture retention was the main effect of the treatment. In the current study, when comparing values for bagged and non-bagged fruit for each cultivar, ‘Brigitta’ showed 4.0 vs. 7.8% weight loss in bagged and non-bagged samples, respectively. For ‘Duke’ blueberries, these values were 5.7% and 16.6% for bagged and non-bagged fruit, correspondingly (Table 4). Surprisingly, even though ‘Duke’ fruit picked at H75 and H100 stages had the highest weight loss after storage, the percentage of visually sound fruit was higher for both stages when
compared to H100+X samples. This observation may have arisen from the stronger positive effect of the bagging procedure in this cultivar (81.8% sound fruit and 5.7% weight loss for bagged vs. 24.5% and 16.6% for non-bagged fruit, respectively). On the other hand, differences in weight loss between cultivars could be partially associated to fruit size; it is known that surface/volume ratio of fruit affects transpiration (Ben-Yehoshua et al., 1983). In our study ‘Duke’ fruit had larger surface/volume ratios (Table 2), especially for the H75 stage, which displayed the highest weight loss. Other possible causes might be related to cuticular waxes, as discussed below.

4.2. Wax triterpenoids vs. weight loss, firmness and softening after storage.

The hydrophobic nature of the cuticle has been considered to confer the fruit an effective barrier against water loss (Martin and Rose, 2014; Lara et al., 2014). However, cuticular wax composition and structure, rather than total wax amount, can also impact water permeability (Riederer and Schreiber, 2001). Parsons et al., (2012) found no strong correlation between pepper water loss rate and total wax levels, but an association was seen with specific wax components. Lleide et al. (2011) reported that the cuticular waxes of the ps mutant tomato fruit, which is highly susceptible to water loss, exhibited an almost complete absence of n-alkanes and aldehydes, and increased percentage of triterpenoid and sterol derivatives, when compared to the wild type specimens. Belge et al. (2014a) reported ratios of n-alkanes to triterpenoids of 0.18 and 0.33 on cuticles of ‘Celeste’ and ‘Somerset’ sweet cherries associated with weight loss values of 15.8 and 7.2% after two weeks of refrigerated storage, respectively. Similar results were found on ‘October Sun’ and ‘Jesca’ peaches, where ratios of 0.31 and 0.65 were related to 5.6 and 3.9% weight loss 5 days after harvest, correspondingly (Belge et al., 2014b).
The wax barrier in fruit cuticles is viewed as being relatively impermeable to gases including water vapor and existing as a cluster of crystalline waxes (mainly n-alkanes), both covering and embedded in a matrix of amorphous material (mostly triterpenoids). Water diffusion is considered to occur mostly in the amorphous fraction, while the crystalline cover would prevent further water transport (Vogg et al., 2004). In this study, four triterpenoids were identified, which represented 35 to 50% of total waxes (Table 3). As reviewed in Lara et al. (2014), published information highlights ursolic and oleanolic acids as the main triterpenoids of many fruit species, while other fruit display mainly triterpenols such as amyrins (tomato, pepper, orange, Asian pear). Lupeol has been reported in pear (Cho et al., 2013), citrus (Lara et al., 2015), tomato, grapes, bell pepper, eggplant and grape fruit (Szakiel et al., 2012). Given the results of our study, further research efforts on a putative relationship between high triterpene amounts, their specific composition, and limited storage potential of blueberry fruit, might help shedding light on this important commercial feature.

The compositional differences in the triterpenoid fraction between ‘Duke’ and ‘Brigitta’ was due to the greater content of α-amyrin in the latter cultivar, as well as to the relative ratio of lupeol to oleanolic and ursolic acid (Fig. S1 and S2). Interestingly, α-amyrin and oleanolic acid share a similar carbon skeleton (Neto, 2010). Among triterpenoid compounds, ursolic acid was highly related to weight loss and softening rates: ‘Duke’, which suffered the highest deterioration rates during postharvest, had 2-4 times higher ursolic acid content than ‘Brigitta’. Additionally, oleanolic acid, which was found to be inversely correlated to softening, was more abundant in ‘Brigitta’. Remarkable maturity-related differences were found for ‘Duke’ in the content of the different triterpenoid compounds identified in this work, while changes were very moderate or non-existent in ‘Brigitta’ fruit (Fig. 1). This might explain, partially, the higher weight loss rates
observed after cold storage in ‘Duke’ samples. Non-bagged fruit lost 16.6% weight with respect to harvest. Regarding maturity stage, H75 and H100 fruit lost 14.4 and 12.4%, respectively. In contrast, limited differences in water loss were observed for ‘Brigitta’ samples as related to bagging or maturity stage (Table 3). Actually, chromatographic analyses revealed the presence of a small amount of additional wax compounds eluting at the beginning of the run, which were not identified in this work (Fig. S1 and S2). These unidentified compounds were more abundant in ‘Brigitta’. Future work should elucidate whether they correspond to n-alkanes, and hence check if n-alkane to triterpenoid ratios are actually higher in this cultivar, which would support a relevant role of this ratio on water loss rates, as suggested for other fruit species (Leide et al., 2011; Parsons et al., 2012; Belge et al., 2014a and b).

Thus, in order to maximize the storage and transport potential of fresh blueberries, a deeper survey of the properties and postharvest behavior of a wider range of cultivars, as well as the effects therein of harvest maturity and cuticle composition, appears advisable for the development of cultivar-specific picking strategies similar to those developed for other fruit (especially apple cultivars).

In conclusion, according to results reported herein, commercial harvest intervals should be narrower for those cultivars showing higher differences between H100 and H100+X fruit. Additionally, the improved firmness retention resulting from the use of a barrier against moisture loss suggests that widespread adoption of some form of vapor barrier be advisable for long-term storage. Beneficial effects of the bagging procedure might be enhanced by the use of MAP for particular cultivars.

The triterpenoid fraction of cuticular waxes of a given cultivar has the potential to play a role in the postharvest behavior of blueberries. This is the first report that characterizes cuticular
composition of fresh blueberries, so further research will be required for better understanding the implications of these differences. Additional cuticle and cutin components, as well as the scar morphology may also have important implications on these aspects, and should be considered in future studies.

References


Juillerat, F. 2014. Cómo llegan nuestros arándanos a destino


Lara, I., Belge, B., Goulao, L. 2015. A focus on the biosynthesis and composition of cuticle in fruits. J. Agric. Food Chem. 2015, 63, 4005-4019. DOI: 10.1021/acs.jafc.5b00013


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Multidisciplinario”, Universidad de Talca. In Spain this work was partially supported by “Fundación Carolina” and “Programa de Doctorado en Ciencia y Tecnología Agraria y Alimentaria”, Universitat de Lleida.
Table 1. Fruit maturity and quality assessments at harvest of ‘Duke’ and ‘Brigitta’ blueberries picked at three different maturity stages [75% blue color and pink button (H75), 100% blue and residing on the plant for a maximum of 2 days (H100), and 100% blue and residing on the plant for 5 to 7 days (H100+X)].

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Maturity stage</th>
<th>Firmness (N)</th>
<th>TSS (%)</th>
<th>TA (% citric ac.)</th>
<th>TSS/TA</th>
<th>EP (ng kg⁻¹ s⁻¹)</th>
<th>RR (μg CO₂ kg⁻¹ s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Duke’</td>
<td>H75</td>
<td>1.76 a</td>
<td>11.6 c</td>
<td>1.02 a</td>
<td>11.5 c</td>
<td>0.32</td>
<td>17.83 b</td>
</tr>
<tr>
<td></td>
<td>H100</td>
<td>1.69 a</td>
<td>13.8 b</td>
<td>0.69 b</td>
<td>20.1 b</td>
<td>0.25</td>
<td>11.61 a</td>
</tr>
<tr>
<td></td>
<td>H100 +X</td>
<td>1.38 b</td>
<td>16.4 b</td>
<td>0.65 b</td>
<td>25.4 a</td>
<td>0.22</td>
<td>16.97 b</td>
</tr>
<tr>
<td>Significance (p)</td>
<td></td>
<td>0.0005</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.5015</td>
<td>0.0003</td>
</tr>
<tr>
<td>‘Brigitta’</td>
<td>H75</td>
<td>1.63 a</td>
<td>12.3 b</td>
<td>1.14 a</td>
<td>10.9 b</td>
<td>0.20</td>
<td>11.49 a</td>
</tr>
<tr>
<td></td>
<td>H100</td>
<td>1.55 a</td>
<td>14.7 a</td>
<td>0.76 b</td>
<td>19.7 a</td>
<td>0.19</td>
<td>6.63 b</td>
</tr>
<tr>
<td></td>
<td>H100 +X</td>
<td>1.31 b</td>
<td>14.7 a</td>
<td>0.64 b</td>
<td>23.5 a</td>
<td>0.16</td>
<td>8.85 b</td>
</tr>
<tr>
<td>Significance (p)</td>
<td></td>
<td>0.0031</td>
<td>0.0066</td>
<td>0.0010</td>
<td>0.0023</td>
<td>0.4265</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

For a given cultivar, different letters within a column represent significant differences (Tukey’s test, p ≤ 0.05).

z Firmness: values represent 4 replicates of 25 fruit each
y TSS: Total soluble solids, values represent 4 replicates of 5 fruit each
x TA: Titratable acidity, values represent 4 replicates of 10 mL juice each
w EP: Ethylene production, values represent 4 replicates of three fruit each
v RR: Respiration rate, values represent 4 replicates of three fruit each
Table 2. Fruit size and cuticular wax content of 'Duke' and 'Brigitta' blueberries picked at three different maturity stages [75% blue color and pink button (H75), 100% blue and residing on the plant for a maximum of 2 days (H100), and 100% blue and residing on the plant for 5 to 7 days (H100+X)].

For a given cultivar, different letters within a column represent significant differences (Tukey's test, \( p \leq 0.05 \)). Values represent the mean of 4 replicates of 25 fruit each.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Maturity</th>
<th>Fruit weight (g)</th>
<th>Fruit diameter</th>
<th>Surface /Volume ratio</th>
<th>Wax content per area (g m(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>'Duke'</td>
<td>H75</td>
<td>1.44 b</td>
<td>13.69 b</td>
<td>9.53 c</td>
<td>5.07 a</td>
</tr>
<tr>
<td></td>
<td>H100</td>
<td>1.72 a</td>
<td>14.65 a</td>
<td>10.07 b</td>
<td>4.76 a</td>
</tr>
<tr>
<td></td>
<td>H100 +X</td>
<td>1.72 a</td>
<td>15.59 a</td>
<td>10.91 a</td>
<td>4.44 b</td>
</tr>
<tr>
<td></td>
<td>Significance (( p ))</td>
<td>0.0000</td>
<td>0.0013</td>
<td>0.0000</td>
<td>0.0437</td>
</tr>
<tr>
<td>'Brigitta'</td>
<td>H75</td>
<td>2.11 b</td>
<td>14.92 b</td>
<td>10.81</td>
<td>4.56 a</td>
</tr>
<tr>
<td></td>
<td>H100</td>
<td>2.21 b</td>
<td>15.12 a</td>
<td>11.38</td>
<td>4.60 a</td>
</tr>
<tr>
<td></td>
<td>H100 +X</td>
<td>2.43 a</td>
<td>16.36 a</td>
<td>11.36</td>
<td>4.24 b</td>
</tr>
<tr>
<td></td>
<td>Significance (( p ))</td>
<td>0.00014</td>
<td>0.0029</td>
<td>0.1734</td>
<td>0.0287</td>
</tr>
</tbody>
</table>
Table 3. Triterpene composition (relative %) of ‘Duke’ and ‘Brigitta’ blueberries picked at three different maturity stages [75% blue color and pink button (H75), 100% blue and residing on the plant for a maximum of 2 days (H100), and 100% blue and residing on the plant for 5 to 7 days (H100+X)].

<table>
<thead>
<tr>
<th></th>
<th>Triterpenoid (%)</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Amyrin</td>
<td>Lupeol</td>
<td>Oleanolic Acid</td>
<td>Ursolic Acid</td>
<td>Total</td>
</tr>
<tr>
<td>Cultivar</td>
<td>Maturity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>'Duke'</td>
<td>H75</td>
<td>Nd</td>
<td>32.7 b</td>
<td>3.9 a</td>
<td>13.3 a</td>
</tr>
<tr>
<td></td>
<td>H100</td>
<td>Nd</td>
<td>34.6 b</td>
<td>3.2 a</td>
<td>9.7 ab</td>
</tr>
<tr>
<td></td>
<td>H100 +X</td>
<td>Nd</td>
<td>43.2 a</td>
<td>1.4 b</td>
<td>5.7 b</td>
</tr>
<tr>
<td></td>
<td>Significance</td>
<td>(p)</td>
<td>0.0035</td>
<td>0.0413</td>
<td>0.0250</td>
</tr>
<tr>
<td>'Brigitta'</td>
<td>H75</td>
<td>6.6</td>
<td>25.1 a</td>
<td>9.8</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>H100</td>
<td>5.8</td>
<td>25.1 a</td>
<td>8.7</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>H100 +X</td>
<td>5.2</td>
<td>15.9 b</td>
<td>9.8</td>
<td>4.1</td>
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<tr>
<td></td>
<td>Significance</td>
<td>(p)</td>
<td>0.2143</td>
<td>0.0031</td>
<td>0.7026</td>
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</tbody>
</table>

For a given cultivar, different letters within a column represent significant differences (Tukey's test, $p \leq 0.05$). Values represent the mean of 3 replicates of 25 fruit each. Nd, non detected.
Table 4. Fruit quality assessments and weight loss of ‘Duke’ and ‘Brigitta’ blueberries picked at three different stages [75% blue color and pink button (H75), 100% blue and residing on the plant for a maximum of 2 days (H100), and 100% blue and residing on the plant for 5 to 7 days (H100+X)], and stored either bagged or non-bagged for 45 days at 0 ºC + 1 day at 18 ºC.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Firmness (N)</th>
<th>TSS (%)</th>
<th>TA (%)</th>
<th>TSS/TA</th>
<th>Sound fruit (%)</th>
<th>Weight loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Duke’ Maturity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H75</td>
<td>1.20 ab</td>
<td>13.6 b</td>
<td>1.11 a</td>
<td>12.8 c</td>
<td>52.9 a</td>
<td>14.4 a</td>
</tr>
<tr>
<td>H100</td>
<td>1.27 a</td>
<td>15.4 a</td>
<td>0.73 b</td>
<td>22.0 b</td>
<td>63.4 a</td>
<td>12.4 a</td>
</tr>
<tr>
<td>H100 +X</td>
<td>1.13 b</td>
<td>16.3 a</td>
<td>0.51 c</td>
<td>34.2 a</td>
<td>42.6 b</td>
<td>6.6 b</td>
</tr>
<tr>
<td>Significance (p)</td>
<td>0.0114</td>
<td>0.0001</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0073</td>
</tr>
<tr>
<td>Bagging (B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bag</td>
<td>1.45 a</td>
<td>14.6 b</td>
<td>0.76</td>
<td>23.5</td>
<td>81.8 a</td>
<td>5.7 b</td>
</tr>
<tr>
<td>No Bag</td>
<td>0.95 b</td>
<td>15.6 a</td>
<td>0.80</td>
<td>22.5</td>
<td>25.4 b</td>
<td>16.6 a</td>
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<td>0.0256</td>
<td>0.3787</td>
<td>0.6204</td>
<td>0.0000</td>
<td>0.0000</td>
</tr>
<tr>
<td>M x B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance (p)</td>
<td>0.6225</td>
<td>0.1055</td>
<td>0.8248</td>
<td>0.2001</td>
<td>0.0865</td>
<td>0.7081</td>
</tr>
<tr>
<td>‘Brigitta’ Maturity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H75</td>
<td>1.56 a</td>
<td>12.0 c</td>
<td>0.94 a</td>
<td>13.8 b</td>
<td>90.8 a</td>
<td>5.5 ab</td>
</tr>
<tr>
<td>H100</td>
<td>1.58 a</td>
<td>14.7 b</td>
<td>0.60 b</td>
<td>24.9 a</td>
<td>89.3 a</td>
<td>7.2 a</td>
</tr>
<tr>
<td>H100 +X</td>
<td>1.06 b</td>
<td>15.9 a</td>
<td>0.60 b</td>
<td>27.8 a</td>
<td>80.3 b</td>
<td>5.0 b</td>
</tr>
<tr>
<td>Significance (p)</td>
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<td>0.0000</td>
<td>0.0000</td>
<td>0.00637</td>
<td>0.3179</td>
</tr>
<tr>
<td>Bagging (B)</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Bag</td>
<td>1.49 a</td>
<td>14.4</td>
<td>0.74</td>
<td>22.4</td>
<td>92.2 a</td>
<td>4.0 b</td>
</tr>
<tr>
<td>No Bag</td>
<td>1.31 b</td>
<td>14.0</td>
<td>0.69</td>
<td>21.9</td>
<td>81.4 b</td>
<td>7.8 a</td>
</tr>
<tr>
<td>Significance (p)</td>
<td>0.0026</td>
<td>0.2345</td>
<td>0.5033</td>
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<td>0.0000</td>
<td>0.0001</td>
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<tr>
<td>M x B</td>
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<tr>
<td>Significance (p)</td>
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<td>0.0000</td>
<td>0.0010</td>
<td>0.7860</td>
<td>0.3207</td>
</tr>
</tbody>
</table>

For a given cultivar or factor, different letters within a column represent significant differences (Tukey’s test, \( p \leq 0.05 \)).

\(^a\) Firmness: values represent 4 replicates of 25 fruit each

\(^b\) TSS: Total soluble solids, values represent 4 replicates of 5 fruit each

\(^x\) TA: Titratable acidity, values represent 4 replicates of 10 mL juice each

\(^w\) Sound fruit and weight loss, values represent 4 replicates of 50 fruit each
Table 5. Linear correlation coefficients (r)\(^z\) between fruit characteristics at harvest and postharvest evaluations (weight loss and softening) of ‘Duke’ and ‘Brigitta’ blueberries picked at three different maturity stages [75% blue color and pink button (H75), 100% blue and residing on the plant for a maximum of 2 days (H100), and 100% blue and residing on the plant for 5 to 7 days (H100+X)], and stored for 45 days at 0 ºC + 1 day at 18 ºC.

<table>
<thead>
<tr>
<th></th>
<th>Fruit quality</th>
<th></th>
<th>Wax compounds (g m(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fruit weight</td>
<td>Surface/ Volume</td>
<td>EP (ng kg(^{-1}) s(^{-1}))</td>
</tr>
<tr>
<td></td>
<td>(g)</td>
<td>Ratio</td>
<td>RR (μg kg(^{-1}) s(^{-1}))</td>
</tr>
<tr>
<td>Weight loss (%)</td>
<td>-0.82(^**)</td>
<td>0.91(^*)</td>
<td>0.78(^{ns})</td>
</tr>
<tr>
<td></td>
<td>-0.51(^{ns})</td>
<td>-0.57(^{ns})</td>
<td>0.17(^{ns})</td>
</tr>
<tr>
<td></td>
<td>0.17(^{ns})</td>
<td>-0.37(^{ns})</td>
<td>0.94(^**)</td>
</tr>
<tr>
<td></td>
<td>0.94(^**)</td>
<td>0.49(^{ns})</td>
<td>0.49(^{ns})</td>
</tr>
<tr>
<td></td>
<td>0.40(^{ns})</td>
<td>---</td>
<td>0.34(^{ns})</td>
</tr>
<tr>
<td>Weight loss (% m(^{-2}))</td>
<td>-0.78(^{ns})</td>
<td>0.89(^*)</td>
<td>0.78(^{ns})</td>
</tr>
<tr>
<td></td>
<td>-0.57(^{ns})</td>
<td>-0.57(^{ns})</td>
<td>0.21(^{ns})</td>
</tr>
<tr>
<td></td>
<td>0.17(^{ns})</td>
<td>-0.41(^{ns})</td>
<td>0.92(^**)</td>
</tr>
<tr>
<td></td>
<td>0.92(^**)</td>
<td>0.47(^{ns})</td>
<td>0.47(^{ns})</td>
</tr>
<tr>
<td>Softening (%)</td>
<td>-0.96(^**)</td>
<td>0.76(^{ns})</td>
<td>0.59(^{ns})</td>
</tr>
<tr>
<td>(firmness loss)</td>
<td>0.76(^{ns})</td>
<td>-0.32(^{ns})</td>
<td>0.10(^{ns})</td>
</tr>
<tr>
<td></td>
<td>-0.21(^{ns})</td>
<td>-0.21(^{ns})</td>
<td>0.94(^**)</td>
</tr>
<tr>
<td></td>
<td>0.94(^**)</td>
<td>0.81(^*)</td>
<td>0.81(^*)</td>
</tr>
<tr>
<td></td>
<td>0.55(^{ns})</td>
<td>---</td>
<td>0.50(^{ns})</td>
</tr>
</tbody>
</table>

\(^z\) n=6

ns, non significant

\(^*\) p ≤ 0.05

\(^**\) p ≤ 0.01
Figure 1. Main triterpene content (g m\(^{-2}\)) in cuticular waxes isolated from ‘Duke’ and ‘Brigitta’ blueberries harvested at three maturity stages. For each cultivar and component, values bearing different letters are significantly different (Tukey’s test, \(p \leq 0.05\)).
Supplementary Material
Click here to download Supplementary Material: Supplementary material.docx