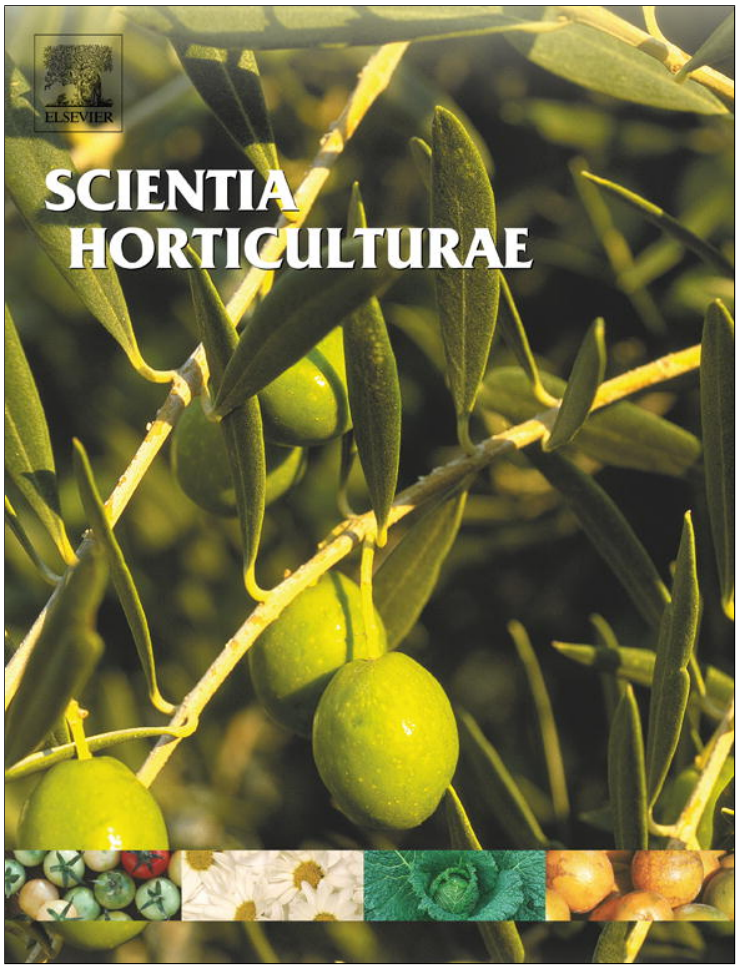

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Berberis buxifolia fruit growth and ripening: Evolution in carbohydrate and organic acid contents

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ABSTRACT

Evolution in the contents of carbohydrates and organic acids of *Berberis buxifolia* fruits during the growth and ripening in different growing seasons (2004/05, 2005/06 and 2007/08), and their correlations with physicochemical variables are presented. Glucose and fructose contents increased during the fruiting period reaching maximum values between 98 and 126 days from full flower phase (*DFFP*). Insoluble and soluble dietary fiber contents attained maximum values between 56 and 70 *DFFP*, to then decrease toward the end of ripening, although soluble dietary fiber during 2005/06 presented a peak later. Citric and malic acid contents increased during the fruiting period being maximum between 56 and 70 *DFFP*, to then decrease toward the end of ripening, although citric acid content during 2007/08 stayed constant from 70 *DFFP*. Oxalic and tartaric acid contents were maximal between 42 and 70 *DFFP*; to then decrease toward the end of the fruiting period. According to the obtained results, *B. buxifolia* fruits can be considered as fruits rich in carbohydrates. Contents of carbohydrates and organic acids during the fruit growth and ripening varied with the specific compound or group considered, i.e., soluble sugars, fibers or organic acid type and might correlate with features of quality of fruit including weight, firmness and color, soluble solids and total titratable acidity, which are responsible for the astringency, texture, taste and color of the fruits.

1. Introduction

Both, carbohydrates and organic acids, which proceed from the photosynthetic assimilation, are of special importance in fruit development and ripening physiology. Carbohydrates are the primary compounds to be metabolically used through the fruit stages. In fact, large quantities of these primary products are immediately converted into other compounds such as organic acids, which are also important in plant metabolism (Kramer and Kozlowski, 1979). Carbohydrates are also important components of the sensorial quality attributes in fruits contributing to their sweetness, flavor and eating quality (Vicente et al., 2009). Sugar content of fruits is determined by both intrinsic (sugar signal and other intrinsic signals) and extrinsic factors (nutrients, plant hormones and physical factors) on sugar transport, metabolism, accumulation and the relationship among them (Zhi et al., 2004). Glucose and fructose are the predominant forms of simple sugars found in fruits, and the relative proportions between them vary, from fruit to fruit and, to a lower extent, in the same fruit according to maturity (Vicente et al., 2009). Soluble dietary fiber includes carbohydrates coming from secondary metabolism like pectins, gums and mucilages, pentosans and other polysaccharides, while insoluble dietary fiber includes cellulose, hemicellulose and lignin. The variation in the dietary fiber content together with the fruit firmness during ripening has been reported by El-Zoghbi (1994). Organic acids, and in special the ratio sugars to acids affects the flavor of fruits. The most abundant acids in fruits are citric and malic acids, although large amounts of tartaric acid occur in *Vitis* fruits (Vicente et al., 2009). In addition, a correlation between environmental factors like temperature and malic acid level has been observed in *Vitis* fruits (Conde et al., 2007).

Horticultural crops are some of the main components of a healthy diet (Vicente et al., 2009), and they have been associated with a reduced risk of chronic diseases (Ruiz et al., 2010), probably due to the high content of carbohydrates such as fibers among other compounds (Lajolo et al., 2001). Indeed, the consumption of

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locally grown, wild or semi wild edible plants has been important for most human cultures and they often contain higher amount of nutrients and bioactive compounds than many cultivated species (Ruiz-Rodríguez et al., 2011). Native patagonian species, like those of *Berberis* L. genus (Orsi, 1984), whose fruits are of economic value (Arena and Curvetto, 2008), have made an important contribution to the food and health of local cultures (Dominguez Diaz, 2010), although only a few species have been evaluated for their agronomic and medicinal potential (Alonso and Desmarchelier, 2006). Carbohydrate and organic acid contents in fruits of *Berberis* have been previously reported for *B. vulgaris* (Pozniakovskíí et al., 2003) and for *B. microphylla* (Ruiz et al., 2010), while in *Berberis buxifolia* only the fruit growth and the evolution of chemical properties together with the antioxidant activity during the fruiting period were previously studied (Arena and Curvetto, 2008; Arena et al., 2012), being soluble solids and soluble solids/total titratable acidity ratio some of the best quality traits for harvesting these fruits. The aim of this work is to study the evolution of carbohydrate and organic acid contents during fruit growth and ripening along different growing seasons, due to compositional changes that occur during ripening affect both the organoleptic and nutritional quality of fruits. Understanding these developmental changes may aid optimization of fruit quality (Forney et al., 2012). The correlation among carbohydrate and organic acid contents with the evolution of the physicochemical variables during fruit growth and ripening as well as with the climatic conditions along the growing season is the hypothesis to be tested.

2. Materials and methods

2.1. Geographic data and climatic parameters

B. buxifolia plants were growing naturally in a representative area located near Ushuaia city, 54° 48′SL, 68° 19′WL, 30 MASL (Tierra del Fuego, Argentina). Values for mean, maximum and minimum air daily temperatures (°C) and rainfall (mm) were collected. These data were recorded using a meteorological station located at the Centro Austral de Investigaciones Científicas (CONICET, Argentina) from October to March for the 2004/05, 2005/06 and 2007/08 growing seasons.

2.2. Plant material and sampling

B. buxifolia plants (six samples of eight plant each, with a mean height of 0.91 ± 0.15 m), were chosen in 2004 for this experiment. Healthy and sun-exposed fruits (50–60 g each sample) were manually and randomly collected every 14 days from November (14 days from full flower phase *DFFP*) to March (126 *DFFP*), during the 2004/05, 2005/06 and 2007/08 growing seasons. The full flowering phase occurred at mid spring for one to two weeks (Arena et al., 2003, 2011), while the end of the fruiting period was on March 17, 7 and 5 for the 2004/05, 2005/06 and 2007/08 growing seasons, respectively. At 126 *DFFP*, only a few fruits remained on the shrubs and at this time they were overripe.

2.3. Fruit growth analysis

The fresh fruit weight and dry fruit weight (placing the fruits in a dryer at 50 °C until constant weight) were recorded on six samples (5 fruits each).

2.4. Soluble solid content and total titratable acidity

Soluble solids were determined in fruit juice using an ATAGO N1-α refractometer with 0–32 °Brix measurement range with 0.2 °Brix increments, and no temperature compensation. Total titratable acidity was measured by titration with 0.1 N NaOH solution. Total titratable acidity was expressed as malic acid/100 g fresh fruit (%). Soluble solids and total titratable acidity were determined on six samples (10–20 g fresh fruit each).

2.5. Anthocyanin content

Anthocyanin content was quantified by the pH differential method (Giusti and Wrolstad, 2001). Samples (5 g) of initially frozen fruits were extracted for 24 h in 50 mL 0.1% HCl–MeOH solution at 4 °C. Aliquots were then diluted from 1:5 to 1:80 to meet the Lambert–Beer's law, with either 0.025 M KCl (pH 1) or 0.4 M sodium acetate buffer (pH 4.5). Absorbance values at 510 and 700 nm were recorded with a Shimadzu 1203 UV–vis spectrophotometer. Anthocyanin content was determined on the basis of a molar extinction coefficient of 26,900 M^{-1} cm^{-1} and a molecular mass of 449.2 for cyanidin 3-glucoside. Anthocyanin content was determined as follow (mg/100 g fresh-frozen fruit weight) = (absorbance × molecular mass × dilution factor × initial volume/ε × sample weight) × 100, where: absorbance = $(A_{510nm}-A_{700nm})_{pH\ 1.0} - (A_{510nm}-A_{700nm})_{pH\ 4.5}$. Anthocyanin content was determined on six samples (5 g fresh fruit each).

2.6. Carbohydrates extraction and analysis

Simple sugars such as fructose and glucose were analyzed by HPLC (Lee et al., 1992). Dry fruit samples were homogenized immediately before analyses. The sample (1 g) was accurately weighed into a 200-mL Pyrex beaker, extracted with ca. 100 mL boiling water, pH 6–8 during 30 min and then kept at 85 °C with continuous stirring on a hot plate for 15 min. Resulting homogenate extract was then cooled to room temperature, made up to 100 mL and filtered through a 0.20-μm membrane filter (Minisart Sartorius or equivalent) before injection. The chromatographic equipment consisted of a Waters 6000A pump system, a Waters injector with a 50-μL sample loop, a refractive index detector (Waters R40) and an integrator (Data Module Waters). For running the HPLC an Aminex HPX-87C (Bio-Rad) anion exchange column was used, and deionized water at 85 °C as mobile phase with a flux rate of 0.6 mL/min was chosen. Reference sugars (glucose, fructose, and saccharose were from Sigma (Carbohydrates kit, N° CAR-11); reference inulin from Sigma (N° I-375).

For total dietary fiber the AOAC 985.29 and 991.43 methods were used (AOAC, 2000). Dry fruit samples (1 g each) were incubated at 100 °C for 15 min with continuous agitation with heat stable α-amylase to obtain gelatinization, hydrolysis and depolymerization of starch. Then they were incubated with protease at 60 °C for 30 min with continuous agitation (to solubilize and depolymerize proteins) and with amyloglucosidase at 60 °C for 30 min with continuous agitation (to hydrolyze starch fragments to glucose). The samples were removed from the shaking water bath and treated with four volumes of ethanol to precipitate soluble fiber and remove depolymerized protein and glucose (from starch). The residue was filtered; sequentially washed with 78% ethanol, 95% ethanol, and acetone; dried; and weighed. One duplicate was analyzed for protein and another was incinerated for 5 h at 525 °C to determine ash. Total dietary fiber was calculated as the weight of the filtered and dried residue minus the sum of % protein and % ash weights in the residue.

Simple sugars and dietary fibers were determined on six samples (1 g dry fruit weight each). All determinations were made by triplicate. The results were expressed as mean value in mg/g of dry weight of fruit.

2.7. Acid organic extraction and analysis

Celite® (40 g) was suspended in distilled water and beds were packed under vacuum on Whatman N° 1 filter paper in Buchner funnels. About 20 g of whole fresh fruits were processed in a blade grinder and a sample of 12.5 g was weighed and then homogenized in a blender during one min with 100 mL distilled water. The obtained mixtures were filtered under vacuum through Celite® beds. The blender and the Buchner funnels were rinsed with distilled water (two fractions of 50 mL) and the liquids were filtered and added to the fruit extracts, and finally extracts volume was adjusted to 250 mL. The obtained extracts were stored at −20 °C until the chromatographic analyses.

Sample preparation for HPLC analysis: An aliquot of 10 mL of aqueous extract was eluted through a Sep-Pak C_{18} disposable cartridge (Waters Associates, Inc.) to remove interference compounds. The cartridge was activated previously with methanol (5 mL) and, distilled water (5 mL), and dried with 10 mL of air. The first fraction of extract (4–5 mL) was discarded and the next one (4–5 mL) was collected and filtered for liquid chromatographic analysis through aqueous 0.45 μm filter (Millipore Co., Bedford).

Organic Acid Standards: the standard solutions were prepared individually at different concentrations (0.06–3 mg/mL) with Milli Q water. Organic acids (D,L-oxalic, L-tartaric, D,L-malic, and citric acids) were obtained from Aldrich Co. (Sigma–Aldrich Chemie, Steinheim).

HPLC system conditions: the analysis of organic acids was performed by an adaptation of AOAC official method 986.13 (AOAC, 2000). A HPLC system equipped with a Waters™ 600 HPLC pump, a high pressure manual injection valve, and a Waters™ 996 photodiode array detector (Millipore Co., Milford) monitored at 225 nm was used. Integration and data acquisition were performed with Millenium 2010 Chromatography software (Millipore Co., Milford). The organic acids were isocratically eluted using a Synergi Hydro-RP 80A column (250 mm × 4.6 mm × 4 μm) (Phenomenex, USA), preceded by an alpha Bond™ C18 guard column (Alltech Associates, Inc.). The mobile phase (0.02 M K_2HPO_4 keep at pH = 2.9 with H_3PO_4 85% (m/m) was filtered through 0.45 μm Millipore membrane-filter (Millipore Co., Bedford) and degassed in ultrasonic bath. Mobile phase was used at a flow rate of 0.7 mL/min and the injection volume of sample was 10 μL. Each sample was injected in duplicate and the results were expressed as mean value in mg/g dry fruit weight. Citric, malic, oxalic and tartaric acids were determined on six samples (20 g fresh fruit each).

2.8. Statistical analysis

Data were statistically evaluated by two way ANOVA. Coefficient correlations were performed between some pairs of variables.

3. Results

3.1. Climatic description

Mean air daily temperature was higher (9.0 °C) during 2004/05 than in 2005/06 and 2007/08 growing seasons (8.3 and 8.6 °C, respectively). The greatest difference in mean air daily temperature (near 3.0 °C) was found in November (Fig. 1) among the growing seasons. Maximum air daily temperatures were higher up to 13.5 °C during 2004/05 than in the following growing seasons, 12.8 and 13.0 °C respectively, as well as minimum air daily temperatures which reached 4.6, 4.4 and 4.6 °C during the 2004/05, 2005/06 and 2007/08 growing seasons, respectively. The maximum rainfall occurred on December for the 2004/05 growing season, on January for the 2005/06 growing season, and on November for the 2007/08 growing season (Fig. 1).

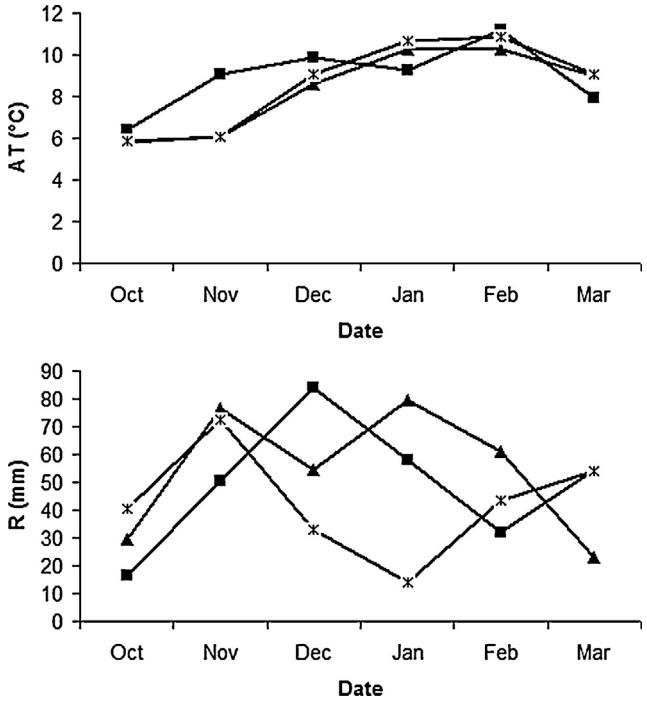

Fig. 1. Climatic data for the experimental region near Ushuaia city, 54° 48′ SL, 68° 19′ WL (Tierra del Fuego, Argentina). Mean air daily temperatures (*AT*) and rainfall (*R*) were recorded from October to March for the 2004/05 (–■–), 2005/06 (–▲–) and 2007/08 (–✳–) seasons.

3.2. Fruit growth analysis

Fresh weight of fruits significantly varied during the fruiting period ($p < 0.001$) and with the growing seasons ($p < 0.001$), with significant interactions between both factors ($p < 0.001$). Fresh fruit weight had higher increments between 14 and 42 *DFFP* in 2004/05 than in the other growing seasons, when the higher increments were observed later (Fig. 2). The fruit growth (fresh weight basis) in the time course from 14 *DFFP* until day 126 shows a typical double sigmoid curve of growth (Fig. 2), as was reported previously for this species (Arena and Curvetto, 2008). In the 2004/05 growing season, the first period of rapid fresh weight increase ended at 42 *DFFP*, followed by a lag period until the day 56 and then by a second period of rapid increases until 70 *DFFP*. Then fresh fruit weight increased slowly until day 84 when it reached its maximum (435.2 mg). Afterwards, fresh fruit weight decreased significantly until the end of the summer. In the following growing seasons, the same general pattern for fruit growth was observed, but in the 2005/06 growing season, the second period of rapid increase in fresh weight had the highest increment, and the decreasing was not so abrupt compared to the 2004/05 growing season, while in 2007/08 the lag period was delayed in time compared to the 2004/05 growing season.

Dry weight of fruits significantly varied during the fruiting period ($p < 0.001$) and with the growing seasons ($p < 0.001$), with significant interactions between both factors ($p < 0.001$). The dry fruit weight evolution closely followed the fresh weight behavior until the maximum fruit biomass was reached (Fig. 2). However, the dry fruit weight did not present significant decreases after this time, particularly in the 2004/05 and 2005/06 growing seasons.

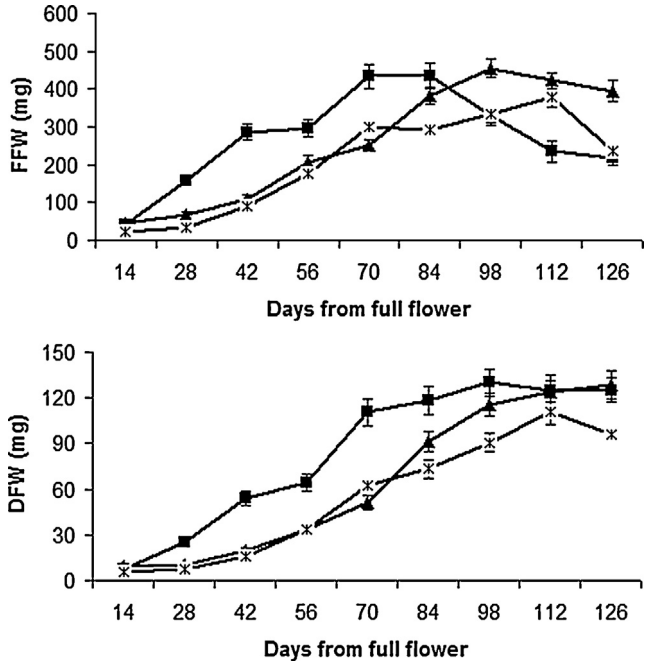

Fig. 2. *B. buxifolia* fruiting growth. Fresh fruit weight (*FFW*) and dry fruit weight (*DFW*) from day 14 to day 126 from the full flower phase for the 2004/05 (–■–), 2005/06 (–▲–) and 2007/08 (–✳–) growing seasons. Bars represent ± standard error of the mean.

3.3. Soluble solid content and total titratable acidity

Soluble solids significantly varied during the fruiting period ($p < 0.001$) and with the growing seasons ($p < 0.001$), with significant interactions between both factors ($p < 0.001$). This parameter increased during the fruiting period being maximum between 112 and 126 *DFFP*, attaining the highest values of 30.7, 25.2 and 30.6 °Brix for the 2004/05, 2005/06 and 2007/08 growing seasons, respectively (Fig. 3). Soluble solids showed the highest increments between 84 and 98 *DFFP* in the 2004/05 growing season, while the highest increments in this parameter were observed later for the subsequent growing seasons. Soluble solids along 2004/05 were higher compared to 2005/06 and 2007/08 growing seasons. The total titratable acidity significantly varied during the fruiting period ($p < 0.001$) and with the growing seasons ($p < 0.001$), with significant interactions between both factors ($p < 0.001$). Total titratable acidity showed its maximum values between 56 and 77 *DFFP*, with 3.6, 4.5 and 6.3% for the 2004/05, 2005/06 and 2007/08 growing seasons respectively, and then it decreased toward the end of ripening (Fig. 3). Total titratable acidity between 56 and 91 *DFFP* in 2004/05 was lower compared to 2005/06 and 2007/08 growing seasons.

3.4. Anthocyanin content

Anthocyanin content in fruit tissue significantly varied during the fruiting period ($p < 0.001$) and with the growing seasons ($p < 0.001$), with significant interactions between both factors ($p < 0.001$), reaching maximum values of 954.7, 743.4 and 762.7 mg/100 g fruit fresh weight between 112 and 126 *DFFP* for the 2004/05, 2005/06 and 2007/08 growing seasons respectively (Fig. 3). Anthocyanin content showed the highest increments among 77 and 98 *DFFP* in the 2004/05 growing season, while the highest increments in this parameter were observed later for the subsequent growing seasons. Anthocyanin content along the 2004/05 was higher compared to 2005/06 and 2007/08 growing seasons.

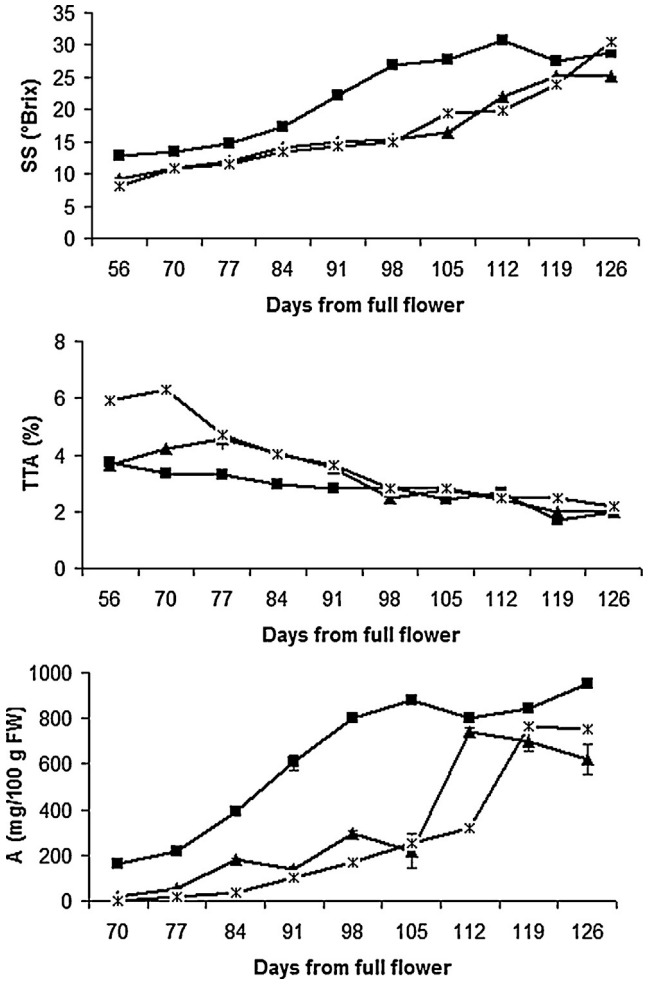

Fig. 3. *B. buxifolia* fruiting chemical properties. Soluble solids (*SS*), total titratable acidity (*TTA*), and anthocyanin content (*A*) from day 56–70 to day 126 from full flower for the 2004/05 (–■–), 2005/06 (–▲–) and 2007/08 (–✳–) growing seasons. FW = fresh weight. Bars represent ± standard error of the mean.

3.5. Carbohydrates analysis

Glucose content significantly varied during the fruiting period ($p < 0.001$) and with the growing seasons ($p < 0.001$), with significant interactions between both factors ($p < 0.001$), reaching maximum values between 98 and 126 *DFFP* of 203.5, 353.9 and 104.2 mg/g dry fruit weight for the 2004/05, 2005/06 and 2007/08 growing seasons respectively (Fig. 4). Fructose content significantly varied during the fruiting period ($p < 0.001$) and with the growing seasons ($p < 0.001$), with significant interactions between both factors ($p < 0.001$), reaching maximum values among 98 and 126 *DFFP* of 149.6, 189.1 and 118.9 mg/g dry fruit weight for the 2004/05, 2005/06 and 2007/08 growing seasons respectively (Fig. 4). Glucose and fructose contents between 42 and 84 *DFFP* along the 2004/05 were higher compared to 2005/06 and 2007/08 growing seasons.

Insoluble dietary fiber content significantly varied during the fruiting period ($p < 0.001$) and with the growing seasons ($p < 0.001$), with significant interactions between both factors ($p < 0.001$), attaining maximum values among 56 and 70 *DFFP* of 488.3, 551.7 and 545.2 mg/g dry fruit weight for the 2004/05, 2005/06 and 2007/08 growing seasons, respectively, and then it decreased toward the end of ripening (Fig. 5). Soluble dietary fiber content significantly varied during the fruiting period ($p < 0.001$) and with the growing seasons ($p < 0.001$), with significant interactions between

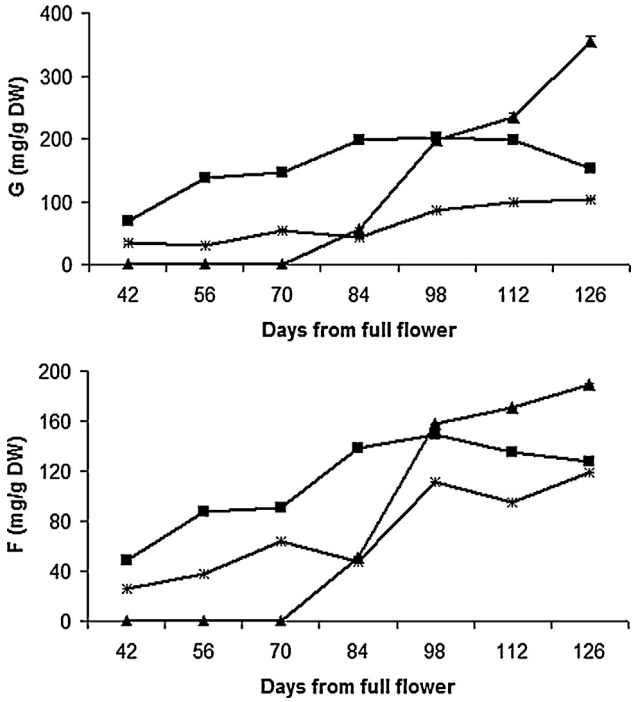

Fig. 4. Glucose (*G*) and fructose (*F*) evolution in *B. buxifolia* fruits from day 42 to day 126 from the full flower phase for the 2004/05 (–■–), 2005/06 (–▲–) and 2007/08 (–✳–) growing seasons. DW = dry weight. Bars represent ± standard error of the mean.

both factors ($p < 0.001$). Soluble dietary fiber content decreased from 176.9 to 47.3 mg/g dry fruit weight during 2004/05 growing season, while this decrease was not so abrupt during the following growing seasons.

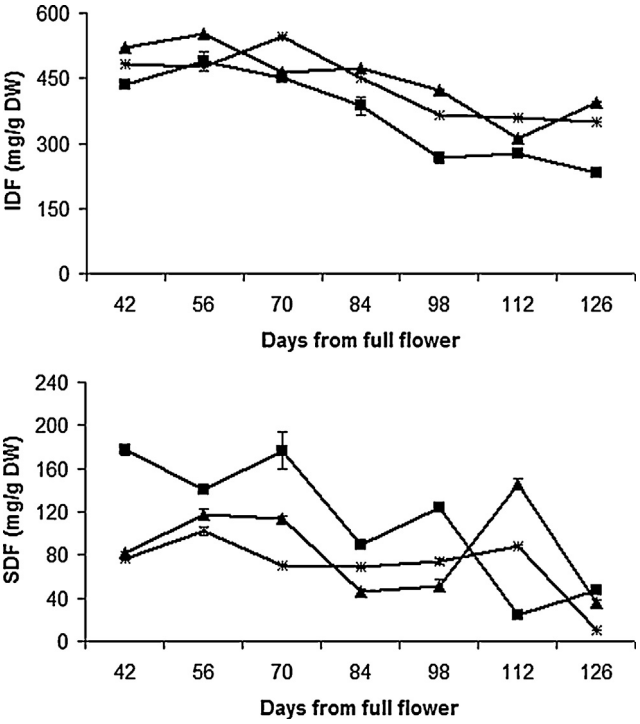

Fig. 5. Insoluble dietary fiber (*IDF*) and soluble dietary fiber (*SDF*) in *B. buxifolia* fruits from day 42 to day 126 from the full flower phase for the 2004/05 (–■–), 2005/06 (–▲–) and 2007/08 (–✳–) growing seasons. DW = dry weight. Bars represent ± standard error of the mean.

3.6. Organic acid analysis

Contents of organic acids significantly changed during the fruiting period ($p < 0.001$), while citric and oxalic acids were the only that changed with the growing season ($p < 0.001$), presenting citric, oxalic and tartaric acids significant interactions between both factors ($p = 0.002$, $p = 0.025$ and $p = 0.035$, respectively).

Citric acid content increased during the fruiting period being maximum 70 *DFFP* for the 2004/05 and 2005/06 growing seasons (15.7 and 6.8 mg/g dry fruit weight, respectively); then this content showed an abrupt decrease toward the end of the fruiting period (Fig. 6). However, citric acid content increased during the fruiting period for the 2007/08, being maximum (4.0 mg/g dry fruit weight) at 112 *DFFP*. Malic acid content increased during the fruiting period being maximum between 56 and 70 *DFFP* (48.8, 81.6 and 66.2 mg/g dry fruit weight for the 2004/05, 2005/06 and 2007/08 growing seasons respectively); then this content decreased toward the end of the fruiting period (Fig. 6). Malic acid content showed an abrupt increase during the fruiting period in the 2005/06 and 2007/08 growing seasons, while this increase was smoother in 2004/05 growing season. Oxalic and tartaric acid contents decreased during the fruiting period, being maximum between 42 and 70 *DFFP* (18.9, 11.3 and 10.2 mg oxalic acid/g dry fruit weight and 10.0, 6.5 and 6.3 mg tartaric acid/g dry fruit weight for the 2004/05, 2005/06 and 2007/08 growing seasons, respectively). Then these contents decreased toward the end of the fruiting period (Fig. 6).

4. Discussion

As a whole the observed changes in the fruit growth are associated with changes in fruit metabolism including also other physiological phenomena at the whole plant level, which can be partially reflected in compositional changes during the fruit ontogeny, as is shown in this case through soluble solids, acidity, anthocyanin, carbohydrates and organic acid contents.

Accumulation patterns of glucose and fructose during the fruiting period varied according to the growing season. In the 2004/05 growing season, the 35.9% of the glucose and fructose content was accumulated during the second period of rapid growth, while in the following growing seasons fruits accumulated the 100.0% and 59.7% of the glucose and fructose content during the same period of rapid growth (Fig. 4). The different behaviors on sugar accumulation patterns could be explained in part by the higher dry matter of the fruits in 2004/05 than in the following growing seasons, which would be related with the great differences in air temperatures found at the beginning of the fruiting period as well as in growing degree-days accumulated among the growing seasons, influencing the earlier fruit sugar accumulation, in concordance with the findings of Zhi et al. (2004). Higher temperatures together with appropriate rainfall might be responsible for the exceptional composition of the fruits in 2004/05 growing season. Considering the role of climate on sugar accumulation, fruits differ in their form to accumulate the carbohydrates, some of them storage before ripening, while other ones continue to accumulate sugars during ripening (Sozzi, 2007). During the first period of rapid growth, most of the sugar imported into the fruits of *B. buxifolia* must be metabolized and so there is little storage, while fruits accumulate most of soluble carbohydrates during ripening due to the mechanism of sugar uptake in the berry may alter at this time. Glucose content was higher than fructose content during the fruiting process, except in the 2007/08 growing season, when since 56 *DFFP*, this ratio was lower than or close to 1. Saccharose was found only in traces as was cited for *Vitis* (Boss and Davies, 2001), *Clidemia rubra* (Gordon et al., 2011) and for *Arbutus unedo* (Ruiz-Rodríguez et al., 2011). Generally, saccharose occurs in berries only in low concentrations

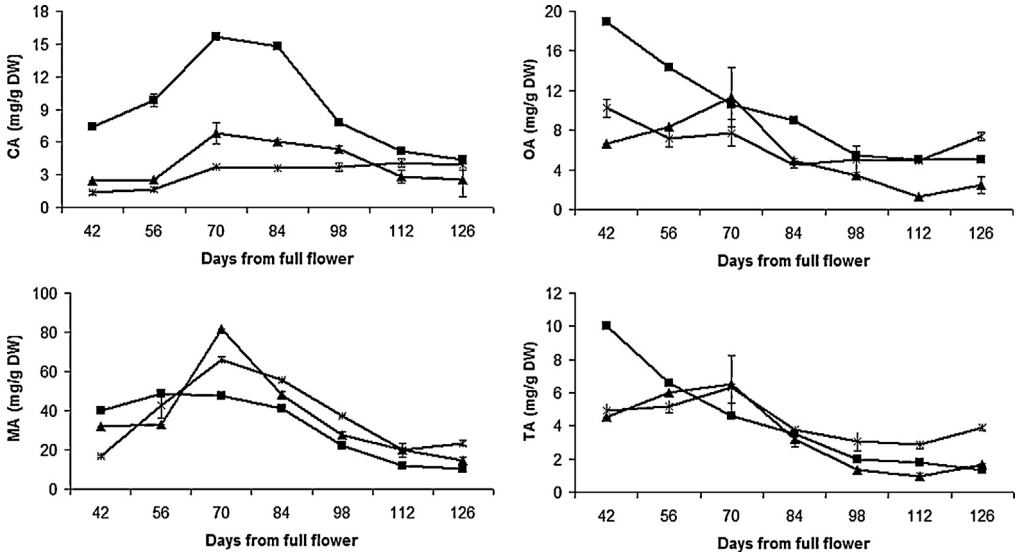

Fig. 6. Citric acid (*CA*), malic acid (*MA*), oxalic acid (*OA*) and tartaric acid (*TA*) in *B. buxifolia* fruits from day 42 to day 126 from the full flower phase for the 2004/05 (–■–), 2005/06 (–▲–) and 2007/08 (–✳–) growing seasons. DW = dry weight. Bars represent ± standard error of the mean.

which can be explained by its transport function inside the plant from the leaves and the immediate inversion to fructose and glucose at place of use (Talcott, 2007). The glucose and fructose contents in *B. buxifolia* fruits were higher than those reported for *B. vulgaris* (Pozniakovskíí et al., 2003), for *B. microphylla* from southern Chile (Ruiz et al., 2010), as well as for fruits of *Clidemia rubra* (Gordon et al., 2011), while were comparable to those found for *Arbutus unedo* (Ruiz-Rodríguez et al., 2011). Comparing the sugar contents with other fruits like cherry (9.9%), strawberry (5.5%), blueberry (6%), raspberry (4.8%), and blackcurrant (5%) (Souci et al., 2008), *B. buxifolia* fruits are rich in simple sugars.

Soluble solids and anthocyanin contents had similar behaviors compared to the simple sugars (the sum of glucose and fructose) in the different growing seasons, as was demonstrated by their positive and significant correlation ($r = 0.7130$, $P = <0.001$ and $r = 0.6996$, $P = <0.001$, respectively). The temporal association of sugar and anthocyanin accumulation may suggest an inter relationship between these two processes. In some plant species it has been shown that anthocyanin structural genes can be regulated by carbohydrates, and it would seem very likely that sugar signaling is involved in berry ripening (Boss and Davies, 2001).

Insoluble dietary fiber increased to a maximum during the first period of rapid growth to then decreases toward the end of the ripening, while soluble dietary fiber presented different behavior according to the growing season. Total dietary fiber in *B. buxifolia* fruits were higher than those reported for berries of *Clidemia rubra* (8.85 g/100 g FW, Gordon et al., 2011), *Ribes nigrum* (6.8 g/100 g FW, Souci et al., 2008), *Gaylussacia brasiliensis* (6.5 g/100 g FW, Bramorski et al., 2011), and for *Vitis* (1 to 3.1%, Pak et al., 2001). According to the fiber contents, *B. buxifolia* fruits can be considered as fruits rich in sugars both available and unavailable, which adds a valuable nutritional profile in the light of the nutritional recommendations of FAO/WHO (FAO/WHO, 2003).

Accumulation patterns of organic acids during the fruiting period varied according to the growing season, e.g., citric acid showed peaks at 70 *DFFP* in the 2004/05 and 2005/06 growing seasons, to then decrease toward the end of the fruiting period, while in the 2007/08 growing season the citric acid content stayed constant from 70 *DFFP* until the end of this period (Fig. 6). Malic acid accumulation presented peaks between 56 and 70 *DFFP* for the 2004/05, 2005/06 and 2007/08 growing seasons. The drop in the content of the sum of the organic acids determined as the dry weight of fruits increase ($r = -0.4038$, $P = 0.013$) and with ripening ($r = -0.7084$, $P = <0.001$), tend to be a general phenomenon in fruits, and agree with the data on total acids measured as titratable acids on percent of fresh weight ($r = -0.6128$, $P \leq 0.000$). Especially in fruits where malic acid predominates (such as apple and grapes), a decrease in total acids is found during ripening (Vicente et al., 2009), while in fruits that mainly contain tartaric, citric and ascorbic acids (strawberry, sweet cherry and mulberry species) the organic acid content increases with ripening (Mahmood et al., 2012). The drop of organic acid content that begins at the onset of ripening is associated with a sudden induction of malate oxidation. The simultaneous initiation of organic acid breakdown and sugar accumulation at the onset of ripening, has led to the suggestion that malic acid may be either transformed to fructose and glucose or used as carbon and energy source for respiration (Conde et al. 2007). In particular, a warm climate is known to result in lower acid concentration in these fruits. This negative temperature correlation with malic acid levels is due to the effect of temperature on the balance between malic acid synthesis and catabolism (Conde et al., 2007; Toldam-Andersen and Hansen, 1997). The marked degradation of malic acid found during ripening in the unusual warm season in 2004/05 growing season may indicate such a basic relationship between temperature and malic acid metabolism also exists in *B. buxifolia*, highlighting the importance of knowing the organic acid profile in this wild species.

5. Conclusions

The accumulation patterns of carbohydrates and organic acids during fruit growth and ripening of *B. buxifolia* varied depending on the specific group or compound considered, i.e., soluble sugars, fibers or type of organic acid, and could be correlated with fruit quality characteristics including fruit weight, firmness and color, soluble solids, total titratable acidity and their ratios, which are responsible for astringency, texture, taste and color. This fact is helpful in the identification of suitable harvesting time as well as for the understanding of the effect of preharvest factors such as light and fertilization on this period. The obtained results are of considerable importance when comparing these values with other species, from a practical point of view when the nutritional value of a fruit has to be examined. Also, a wide variability in carbohydrate and organic acid accumulation was found in different growing

seasons highlighting the need to analyze many different samples from different seasons and its relation with the growing season climate, to provide reliable data about chemical composition of wild fruits. Future research on carbohydrate and organic acid contents on different populations will present a great opportunity for genetic improvement of barberries through breeding programs.

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