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Berberis buxifolia fruiting: Kinetic growth behavior and evolution of chemical properties during the fruiting period and different growing seasons

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ABSTRACT

This work studied the growth of *Berberis buxifolia* fruits and some of their chemical attributes during the fruiting period and different growing seasons (2004/2005, 2005/2006, 2006/2007) from November (14 days from full flower phase) till March (126 days from full flower phase) for plants growing in a natural environment near Ushuaia city (Tierra del Fuego, Argentina). *B. buxifolia* fruit growth and composition presented significant changes during the fruiting period and the studied growing seasons. Fresh and dry weight of *B. buxifolia* fruits exhibited a typical double sigmoid curve. The first period of rapid growth was from full flower phase till 42–56 days after, while the second phase of rapid growth began around the 56–70 days from full flower and ended approximately 4 months later. On a dry weight basis the maximum fruit biomass (119.5 mg) was reached 112 days after full flower while maximum fresh weight fruit biomass (424.3 mg) occurred by day 84. Evolution of fruit growth was related with the compositional changes evaluated. By day 126 from full flower, soluble solids (24.9°Brix) and anthocyanin concentration (761.3 mg/100 g fruit fresh weight) were at their maximum values, while at this time the total titratable acidity was at a minimum value (2.56%). The results obtained not only contributes to the knowledge of the quantitative content of anthocyanin, a metabolite with nutraceutical value but, gives some tools for the definition of the optimal harvest time of *B. buxifolia* fruits, what it is important for fruit destination.

1. Introduction

In spite of the well known importance of wild flora as sources of food and medicinal substance, only a few species have been evaluated for their agronomic and medicinal potential (Iriondo, 2001). Hence, more studies on the wild flora diversity and on the agronomic and medicinal potential of these plant species are still needed (Arena and Vater, 2005). Attention is particularly focused on many of the small fruits that are now considered as source of essential organic and inorganic nutrients and metabolic regulation factors, but also considered for their nutraceutical properties as functional foods, i.e. foods containing specific metabolites that give additional health benefits (Henriques et al., 2004; Kuskoski et al., 2005). Soft fruits of *Ribes*, *Rubus* and *Vaccinium* cultivated species are an excellent source of natural products such as pigments (Flores Cantillano, 2004; Henriques et al., 2004) with antioxidant properties (Deighton et al., 2002). The small reddish fruits also occupy an important place in the molecular medicine approach of foods against illnesses, particularly against cancer. Béliveau and Gingras (2005) point out the anti-cancer properties of different phytochemicals associated with small fruits, in particular elagic acid, anthocyanidines, and proanthocyanidines.

The *Berberis* L. genus is well represented in Patagonia by 16 species of native shrubs (Orsi, 1984; Bottini et al., 1993), with a large distribution from Neuquén to Tierra del Fuego (Job, 1942; Orsi, 1984). At present, commercial orchards of *Berberis* are being planned, as it represents an attractive potential crop, first, because its black-blue fruits can be consumed fresh, in marmalades and jams (Orsi, 1984; Bottini et al., 1993), in non-alcoholic beverages, and in ice creams. Second, because it is an important source of alkaloids, i.e. berberines, and phenolic compounds such as anthocyanins, which possess medicinal and tinctorial application (Pomilio, 1973; Shaffer, 1985; Fajardo Morales et al., 1986; Fajardo Morales, 1987).

Berberis buxifolia Lam. is an evergreen, spiny and erect shrub up to 4 m high, often growing in coastal scrub, *Nothofagus* forest margins and clearings, moister areas in grass steppes, and along streams and rivers (Moore, 1983). *B. buxifolia* can be propagated through seeds (Arena and Martínez Pastur, 1994), rhizomes (Arena and Martínez Pastur, 1995; Arena et al., 1998) and in vitro culture

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(Arena et al., 2000). The phenology, growth and fruit production were studied in a natural population of *B. buxifolia* (Arena et al., 2003).

The study of the evolution of fruit size and weight, in correlation with components such as soluble solids, and those giving acidity and pigments can contribute in the establishment of the nutraceutical value at a particular stage of maturity (Gonzalez-San Jose et al., 1990) of B. *buxifolia* fruits. Hence, the aim of this work was to study the growth behavior during the fruiting period of *B. buxifolia* and during different growing seasons, and also to study some of their chemical attributes such as soluble solids, acidity, and anthocyanin content, on plants chosen from a natural environment of Tierra del Fuego (Argentina). The information obtained through this study could be of value in defining the optimal time for harvesting *B. buxifolia* fruits according to their future use, while contributing to the knowledge of their nutraceutical properties through the evaluation of the quantitative content of anthocyanin.

2. Material and methods

2.1. Geographic data and climatic parameters

B. buxifolia plants were studied from an area located near Ushuaia city, 54° 48′ SL, 68° 19′ WL (Tierra del Fuego, Argentina). Climatic data were collected for maximal, minimal, and mean air daily temperatures (°C), mean ambient relative humidity (%), and cumulative rainfall (mm). Data were recorded by a Meteorological Station located at the Centro Austral de Investigaciones Científicas (CONICET, Argentina) from October to March for the 2004/2005, 2005/2006 and 2006/2007 seasons. Cumulative growing degree-days (Souza, 1990), were calculated as the sum of mean daily temperatures above 0 °C from day 0 until 126 days after the full flower phase for the 2004/2005, 2005/2006 and 2006/2007 seasons.

2.2. Plant material, samplings, and measurements

Sun exposed fruits (200 g) were manually collected from the same *B. buxifolia* plants ($n = 50$, with a mean height of 0.91 ± 0.15 m), growing naturally in association with *Chiliotrichium diffusum*, from November (14 days from full flower phenological phase) to March (126 days from full flower phenological phase), during three growing seasons: 2004/2005, 2005/2006 and 2006/2007. The following fruit parameters were recorded and evaluated: fresh weight, dry weight, fruit dry weight as percentage of fresh weight, equatorial and polar fruit diameters (using a digital calipter Mitutoyo Model 500-196, 150 mm × 6 in.–0.01 mm × 0.0005 in.), percentage of fruit surface with purple color, fruit firmness (using a digital penetrometer Wagner Instruments Model FDI 2 [0.001 a 1 kgf], with tips of 1 mm diameter), seed fresh weight, seed dry weight, seed dry weight as percentage of fresh weight, seed number, seed dry weight/fruit dry weight ratio and percentage of fresh seeds with brown color.

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 manual titration equipment and a pH-meter, using a 0.1 N NaOH solution. Total titratable acidity was expressed as malic acid, the most abundant organic acid in *B. buxifolia* fruits. Soluble solids/total titratable acidity ratio and initial pH were also recorded.

Anthocyanin quantification was performed by the pH differential method of 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. Then, aliquots were diluted from 1:5 to 1:80 with either a 0.025 M KCl (pH 1) or 0.4 M sodium acetate (pH 4.5) buffer. Absorbance measurements were made at 510 and 700 nm with a Shimadzu 1203 UV–vis. spectrophotometer. Anthocyanin fruit tissue content was determined on the basis of a molar extinction coefficient of 26,900 and a molecular weight of 449.2 for cyanidin 3-glucoside. Values were expressed in terms of mg of anthocyanin/100 g of fresh-frozen fruit.

Table 1
Climatic data for maximal (MAX), minimal (MIN), and mean (MEA) air daily temperatures (°C), mean ambient relative humidity (%) (HUM), and cumulative rainfall (mm) (RAI) from October to March for the 2004/2005, 2005/2006 and 2006/2007 seasons

	MAX	MIN	MEA	HUM	RAI	AGD
Growing season						
2004/2005	13.5	4.6	9.0	74.9	295.6	1243.4
2005/2006	12.8	4.4	8.3	90.6	324.4	1122.4
2006/2007	12.9	4.4	8.5	70.2	300.0	1101.7

Accumulative growing degree-days (AGD) were calculated as the sum of mean daily temperatures above 0 °C from day 0 until 126 days after the full flower phase for the 2004/2005, 2005/2006 and 2006/2007 seasons.

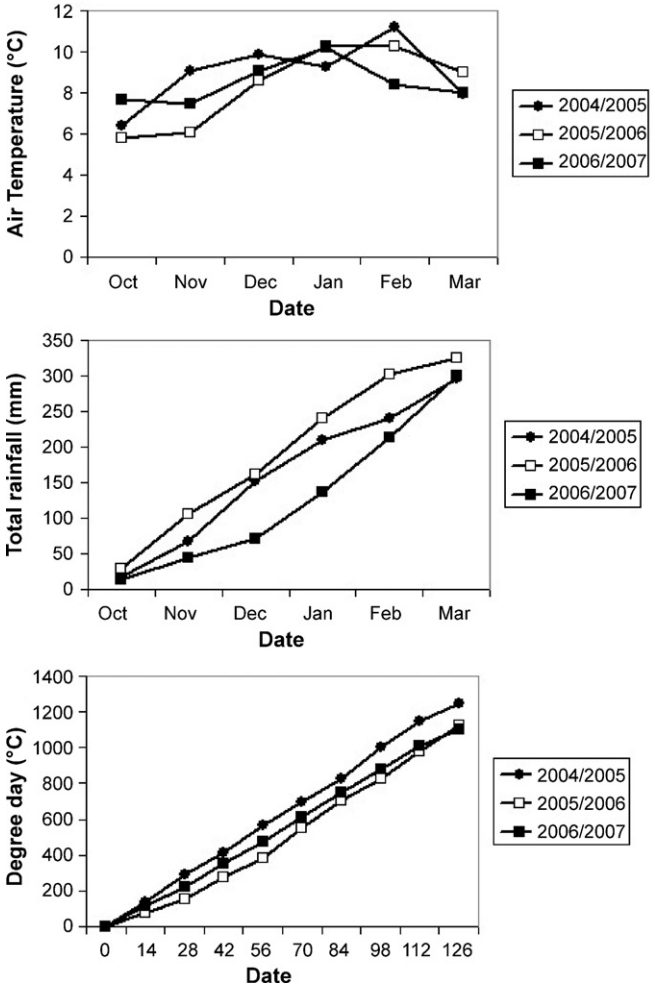

Fig. 1. Climatic data for the experimental region near Ushuaia city, 54° 48′ SL, 68° 19′ WL (Tierra del Fuego, Argentina). Mean air daily temperatures, and cumulative rainfall were recorded from October to March for the 2004/2005, 2005/2006 and 2006/2007 seasons. Cumulative growing degree-days were recorded from day 0 until 126 days after full flower phase for the 2004/2005, 2005/2006 and 2006/2007 seasons.

Anthocyanin fruit content (mg/100 g fruits) = (A × molecular weight × dilution factor × initial volume/ε × sample weight) × 100

with A (absorbance) = $(A_{510\,nm} - A_{700\,nm})_{pH\,1.0} - (A_{510\,nm} - A_{700\,nm})_{pH\,4.5}$

Data were subjected to an analysis of variance, and means were then separated using the Tukey multiple range test at $p \leq 0.05$.

3. Results

3.1. Climatic description

Mean daily temperature was higher (9.0 °C) during 2004/2005 than in 2005/2006 and 2006/2007 growing seasons (8.3 and 8.5 °C, respectively) (Table 1). The greatest differences in mean daily temperatures (near 3.0 °C) were found in November and February (Fig. 1A) among the studied growing seasons. Maximal daily temperatures were higher up to 13.5 °C during 2004/2005 than in the following growing seasons, 12.8 and 12.9 °C, respectively, as well as minimal daily temperatures which reached 4.6, 4.4 and 4.4 °C during the 2004/2005, 2005/2006 and 2006/2007 growing seasons, respectively. Mean ambient relative humidity were 74.9, 90.6, and 70.2% for the 2004/2005, 2005/2006 and 2006/2007 growing seasons, respectively. Total rainfall reached a maximum value of 324.4 mm in 2005/2006, 295.6 mm in 2004/2005, and 300.0 mm in 2006/2007 (Fig. 1B). The maximum rainfall occurred in December for the 2004/2005 growing season, January for the 2005/2006 growing season, and February and March for the 2006/2007 growing season. Cumulative growing degree-days were highest in the 2004/2005 growing season (1243.4 °C) compared to the following seasons (Fig. 1C). Growing degree-days accumulated faster in 2004/2005 than in the other growing seasons, i.e. at 70 days from full flower, growing degree-days were higher, 695.40 °C viz a viz 548.40 and 611.20 °C for the 2005/2006 and 2006/2007 growing seasons, respectively.

3.2. Changes in fruit growth and chemical properties along the fruiting period and among different growing seasons

As expected, the fresh weight of fruits significantly varied during the fruiting period (Table 2), increasing with an average relative growth rate of 15.3% per day (g FW/100 g FW/day) from day 14 after full flower phase, to obtain the highest biomass of 424.3 mg by day 84 from full flower phase, and from this value a decrease averaging 0.54% per day in fresh biomass occurred till the end of the growing season was observed.

The dry fruit weight and dry fruit weight as percentage of fresh weight significantly changed during the fruiting period and each growing seasons (Table 2). These parameters increased from day 14 in the full flower phase at an average rate of 15.7% per day until day 112 when they reached an average biomass of 119.4 mg per fruit, and in the case of dry fruit weight as percentage of fresh weight by day 126 reaching a maximum value of 40.6%. The dry fruit weight (84.5 mg) and dry fruit weight as percentage of fresh weight (31.7%) were significantly higher in 2004/2005 than in the other growing seasons. The equatorial and polar fruit diameters were affected by the fruiting period and the growing season (Table 2). These parameters increased from day 14 of the full flower phase to the maximum values of 9.6 and 8.9 mm for equatorial and polar fruit diameters, respectively on days 84 and 98, and then decreased toward the end of the growing season. The equatorial (8.2 mm) and polar (8.3 mm) fruit diameters were significantly higher in 2004/2005 compared with other years. The percentage of fruit surface with purple color significantly changed during the fruiting period ($F = 67.18$, $p = 0.000$) and with the growing season ($F = 4.32$, $p = 0.013$). The percentage of fruit surface with purple color was close to 40% by day 14 after full flower phase, with the highest increments between 70 and 84 days after full flower phase (64–85%), and 100% of the fruit surface with purple color by day 112 after full flower phase. Fruit firmness was significantly different during the fruiting period ($F = 28.30$, $p = 0.000$), being maximal, i.e. 0.35 and 0.38 kgf by days 28 and 42 after full flower phase, respectively. Firmness decreased towards the end of the

Table 2
Means values of ANOVA analyzing fruit growth of *Berberis buxifolia* considering days from full flower and growing season as main factors, and fresh fruit weight (mg) (FFW), dry fruit weight (mg) (DFW), dry fruit weight as percentage of fresh weight (%) (DFWP), equatorial fruit diameter (mm) (EFD) and polar fruit diameter (mm) (PFD) as dependent variables ($n = 20$)

Main effects	FFW	DFW	DFWP	EFD	PFD
A = Days from full flower					
14	35.76f	7.21e	20.86f	3.88f	5.97d
28	97.74e	16.29de	17.75f	5.30e	6.91c
42	166.77d	31.06cd	18.90f	6.59d	7.40b
56	206.10d	40.02c	18.86ef	7.28c	7.78b
70	364.87bc	87.38b	23.27de	8.98b	8.56a
84	424.30a	112.82a	26.76d	9.63a	8.73a
98	399.15ab	114.31a	30.82c	9.48ab	8.94a
112	352.46bc	119.44a	36.61b	9.14ab	8.74a
126	323.92c	116.33a	40.59a	8.97b	8.56a
$F(p)$	95.86(0.000)	147.39(0.000)	101.95(0.000)	226.67(0.000)	99.40(0.000)
B = Growing season					
2004/2005	270.43	84.46a	31.66a	8.24a	8.29a
2005/2006	259.26	64.91b	22.62b	7.49b	7.95b
2006/2007	260.66	65.59b	23.85b	7.36b	7.62c
$F(p)$	0.55(0.576)	23.46(0.000)	93.29(0.000)	37.11(0.000)	33.07(0.000)
Interactions $F(p)$					
$A \times B$	14.17(0.000)	5.56(0.000)	27.63(0.000)	10.98(0.000)	9.12(0.000)

$F(p)$ = F statistic and probability at $p = 0.05$. Values followed by different letters in each column and for each factor are significantly different with Tukey multiple range test at $p < 0.05$.

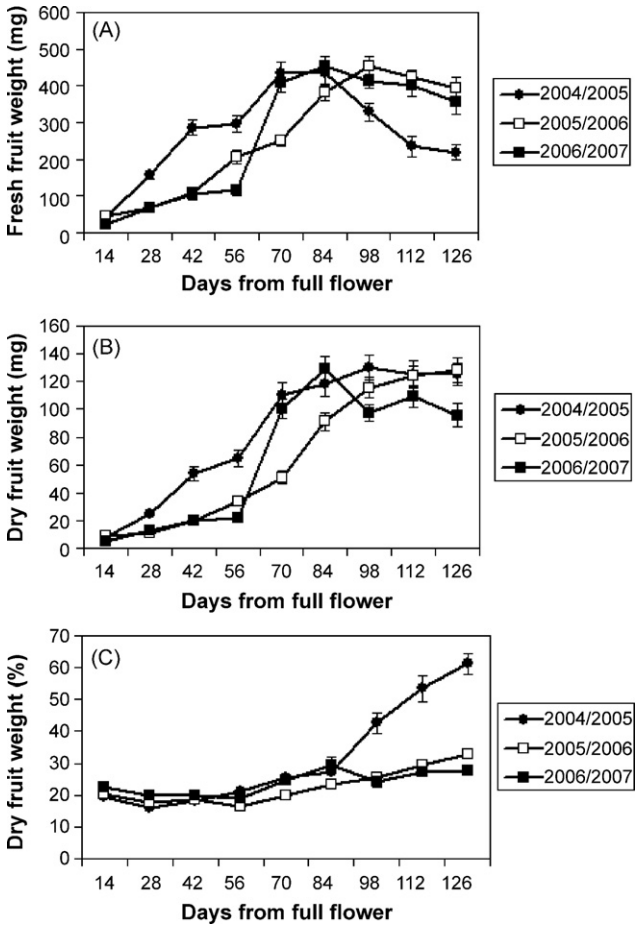

Fig. 2. *Berberis buxifolia* fruiting growth. Fresh and dry fruit weights and dry fruit weight as percentage of fresh weight from day 14 until day 126 after the full flower phase for three growing seasons: 2004/2005, 2005/2006 and 2006/2007. Error bars represent ± S.E.

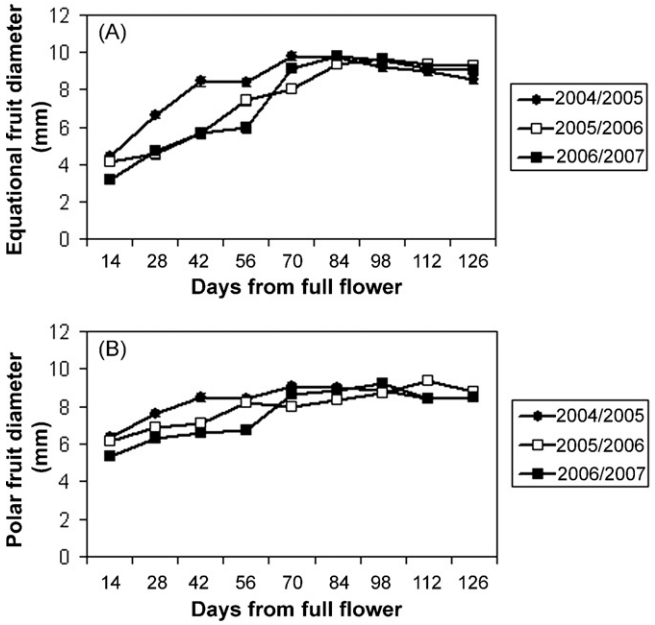

Fig. 3. *Berberis buxifolia* fruiting growth. Equatorial and polar fruit diameters of fruits from day 14 to day 126 after the full flower phase, for three growing seasons: 2004/2005, 2005/2006, and 2006/2007. Error bars represent ± S.E.

fruiting period with a value of 0.14 kgf by day 126 after full flower phase.

Significant interactions were found in all the described parameters in Table 2, mainly due to different value increments between the main factors and differences between combinations (Fig. 2), e.g. fresh and dry fruit weight had higher increments between 14 and 42 days from full flower phase in 2004–2005 than in the other growing seasons.

Fresh weight of fruits had a visible separation into two subsequent periods of rapid increase (Fig. 2A). In the 2004/2005 growing season, the first period of rapid fresh weight increase ended at 42 days from the full flower phase, followed by a lag period until 56 days from the full flower phase and then by a second period of rapid increase until 70 days from the full flower phase. Then fruit fresh weight increased slowly until day 84 when it reached its maximum. Afterwards, fruit fresh 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 2005/2006 growing season, the lag period was delayed in time compared to the 2004/2005 growing season, while in 2006/2007, the second period of rapid increase in fresh weight had the highest increment, and the decreasing was not so abrupt compared to the 2004/2005 growing season.

The dry fruit weight evolution closely followed the fresh weight behavior until the maximum fruit biomass was reached (Fig. 2B). However, the dry fruit weight did not present significant decreases after this time, particularly in the 2004/2005 and 2005/2006 growing seasons. For all the studied seasons, the dry fruit weight as percentage of fresh weight had significant increments from day 56 after the full flower phase, but in the case of the 2004/2005 season, this increment occurred very abruptly from day 84 until day 126 after full flower phase (Fig. 2C). As a whole, the equatorial and polar fruit diameters followed the same behavioral pattern as the fruit weight did, particularly until the attaining the maximal values, as is shown in Fig. 3A and B.

The fresh and dry seed weight significantly varied during the fruiting period and with growing seasons (Table 3). The highest values (110.6 and 52.5 mg for the fresh and dry weight, respectively) were obtained 98 days from full flower phase, decreasing significantly towards the end of the fruiting period. The fresh and dry seed weight, 86.8 and 41.6 mg, respectively, were significantly higher in 2004/2005 than in the other growing seasons. The dry seed weight as percentage of fresh weight significantly varied during the fruiting period and with growing season (Table 3). It increased during the growing season being at a maximum (57.5%) by day 126 from the full flower phase. Dry seed weight was significantly higher (46.3%) in 2005 than the other growing seasons. Seed number significantly varied during the fruiting period and with the growing season (Table 3). After 42 days from the full flower phase, seed number was maximum (10), then decreased during the remaining growing season, due to not all the initial seeds grew during the fruiting period. Seed number was significantly higher (9) in 2006/2007 than in the other growing seasons. Dry seed weight/dry fruit weight ratio also significantly varied during the fruiting period and with the growing season (Table 3); after 84 days from the full flower phase, this ratio was maximum (48.2), decreasing toward the end of the growing season. This ratio was significantly higher (43.1) in 2004/2005 than in the other seasons. As expected, the percentage of fresh seed with brown color significantly changed during the fruiting period ($F = 149.60$, $p = 0.000$). All seeds were green until 56 days after the full flower phase, and 37.5% of them began to show a brown color by day 70 after that phase; 14 days later 80% of seeds were brown.

Table 3
Means values of ANOVA analyzing fruit growth of *Berberis buxifolia* considering days from full flower and growing season as main factors, and fresh seed weight (mg) (FSW), dry seed weight (mg) (DSW), dry seed weight as percentage of fresh weight (%) (DSWP), seed number (SN) and dry seed weight/dry fruit weight ratio (DSW/DFW) as dependent variables ($n = 20$)

Main effects	FSW	DSW	DSWP	SN	DSW/DFW
A = days from full flower					
28	17.93e	4.50e	26.35d	9.01abc	22.54e
42	44.35d	12.27e	28.80d	9.86a	29.69d
56	67.30c	21.98d	30.21d	9.10ab	41.56bc
70	86.43bc	36.04c	40.45c	8.50bc	46.21ab
84	101.70ab	47.83ab	47.13b	7.75cd	48.18a
98	110.61a	52.50a	48.23b	7.21de	44.73ab
112	91.99ab	50.38a	55.84a	6.95de	42.53ab
126	67.87c	39.40bc	57.47a	6.20e	36.26c
F(*p*)	39.18(0.000)	62.22(0.000)	86.10(0.000)	18.38(0.000)	38.95(0.000)
B = growing season					
2004/2005	86.77a	41.56a	46.27a	7.62b	43.07a
2005/2006	70.69b	31.16b	40.19b	8.05ab	37.72b
2006/2007	63.11b	26.61c	38.98b	8.54a	36.10b
F(*p*)	16.76(0.000)	30.21(0.000)	22.98(0.000)	6.34(0.001)	18.57(0.000)
Interactions *F*(*p*)					
$A \times B$	8.13(0.000)	4.38(0.000)	4.08(0.000)	1.51(0.1019)	5.39(0.000)

F(*p*) = *F* statistic and probability at $p = 0.05$. Values followed by different letters in each column and for each factor are significantly different with Tukey multiple range test at $p < 0.05$.

Significant interactions were found between the variables listed in Table 3, except for the seed number, mainly due to different value increments between the main factors and differences between combinations (Fig. 4A and B), e.g. fresh and dry weight of seed had different behaviors between growing seasons, particularly between 2005/2006 and the other two seasons. Both, fresh and dry seed weight presented higher increments near 98 days after full flower phase in the 2005/2006 season viz a viz the other ones.

Soluble solids significantly varied during the fruiting period and with the growing seasons (Table 4). This parameter increased during the fruiting period being maximum (24.9°Brix) by day 126 from the full flower phase. The soluble solids were significantly higher (22.2°Brix) in 2004/2005 than in the other seasons (16.5 and 14.7, respectively). The total titratable acidity significantly varied during the fruiting period and with the growing seasons (Table 4). This parameter had its maximum value 77 days from the full flower phase, and then decreased towards the end of ripening. The total titratable acidity were significantly higher in 2005/2006 and 2006/2007 (3.3% and 3.4%, respectively) compared to 2004/2005 (2.8%). As expected, the soluble solids/total titratable acidity ratio significantly varied during the fruiting period and with growing season (Table 4). This relationship increased along the fruiting period being maximum (12.0) by day 119 from full flower phase, and it was significantly higher (8.7) in 2004/2005 than in the other growing seasons.

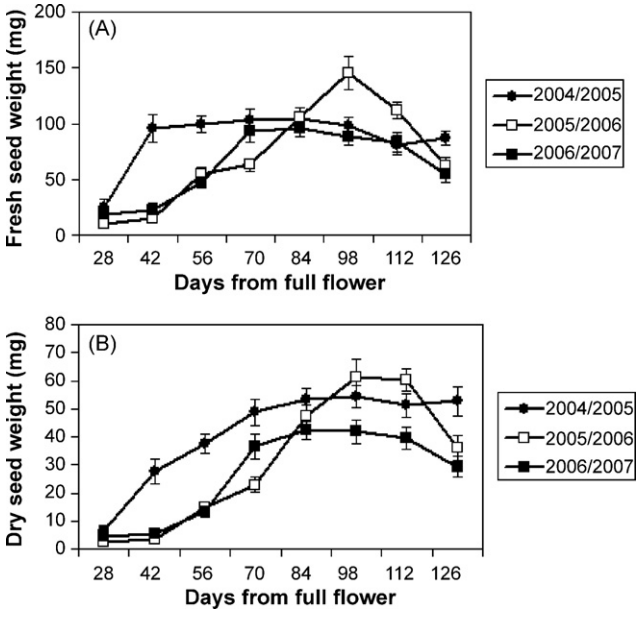

Fig. 4. *Berberis buxifolia* seed weight. Fresh and dry seed weight from day 28 to day 126 from the full flower phase during three growing seasons: 2004/2005, 2005/2006, and 2006/2007. Error bars represent ± S.E.

The initial pH significantly varied during the fruiting period and with the growing season (Table 4). This parameter increased during the fruiting period being maximum (3.1) by day 126 from the full flower phase. The initial pH was significantly higher (3.0) in 2004/2005 than in the other growing seasons.

The anthocyanin fruit tissue concentration significantly varied during the fruiting period and with the growing seasons (Table 4). This parameter increased during the fruiting period being maximum (761.3 mg/100 g fresh fruit weight) by day 126 from the full flower phase. The anthocyanin content was significantly higher (629.5 mg/100 g fresh fruit weight) in 2004/2005 than in the two next seasons (330.2 and 276.7, respectively).

Significant interactions were found between the variables listed in Table 4, except for the total titratable acidity, mainly due to different value increments between the main factors and differences between combinations (Fig. 5A–C), e.g. soluble solids showed the highest increments among 84 and 98 days after the full flower phase in the 2004/2005 growing season, while the highest increments in this parameter were observed later for the subsequent growing seasons. The soluble solids/total titratable acidity ratio increased significantly at the end of the 2004/2005 and 2005/2006 growing seasons, while presenting constant increments in the 2006/2007 season. The period of rapid increase of anthocyanin concentration was earlier in 2004/2005 than in the other growing seasons.

Table 4
Means values of ANOVA analyzing fruit composition of *Berberis buxifolia* considering days from full flower and growing season as main factors, and soluble solids (° Brix) (SS), total titratable acidity (%) (TTA), soluble solids/total titratable acidity relation (RATIO), initial pH (pH), and anthocyanin concentration (mg anthocyanin/100 g fresh fruit) (ANTH) as dependent variables ($n = 6$)

Main effects	SS	TTA	RATIO	pH	ANTH
A = days from full flower					
56	9.38j	3.88ab	2.46f	2.93d	–
70	12.62i	3.97a	3.25ef	2.87ef	80.60f
77	13.71h	4.04a	3.51ef	2.85fg	113.44f
84	15.78g	3.79ab	4.26de	2.81g	222.20e
91	17.10f	3.29abc	5.33d	2.92de	319.29d
98	18.55e	2.76cde	6.70c	2.99c	441.07c
105	19.97d	3.05bcd	7.32c	3.05b	457.39c
112	22.77c	2.55cde	8.67b	3.08b	660.18b
119	23.52b	2.06e	11.99a	3.05b	653.58b
126	24.88a	2.56de	11.24a	3.14a	761.30a
F(*p*)	994.34(0.000)	14.47(0.000)	198.97(0.000)	106.03(0.000)	334.19(0.000)
B = growing season					
2004/2005	22.21a	2.78b	8.73a	3.04a	629.49a
2005/2006	16.52b	3.31a	6.03b	2.89c	330.18b
2006/2007	14.75c	3.44a	4.66c	2.98b	276.69c
F(*p*)	2286.2(0.000)	10.92(0.000)	256.58(0.000)	169.51(0.000)	616.54(0.000)
Interactions *F*(*p*)					
$A \times B$	144.61(0.000)	1.96(0.050)	16.95(0.000)	26.41(0.000)	29.78(0.000)

F(*p*) = *F* statistic and probability at $p = 0.05$. Values followed by different letters in each column and for each factor are significantly different with Tukey multiple range test at $p < 0.05$.

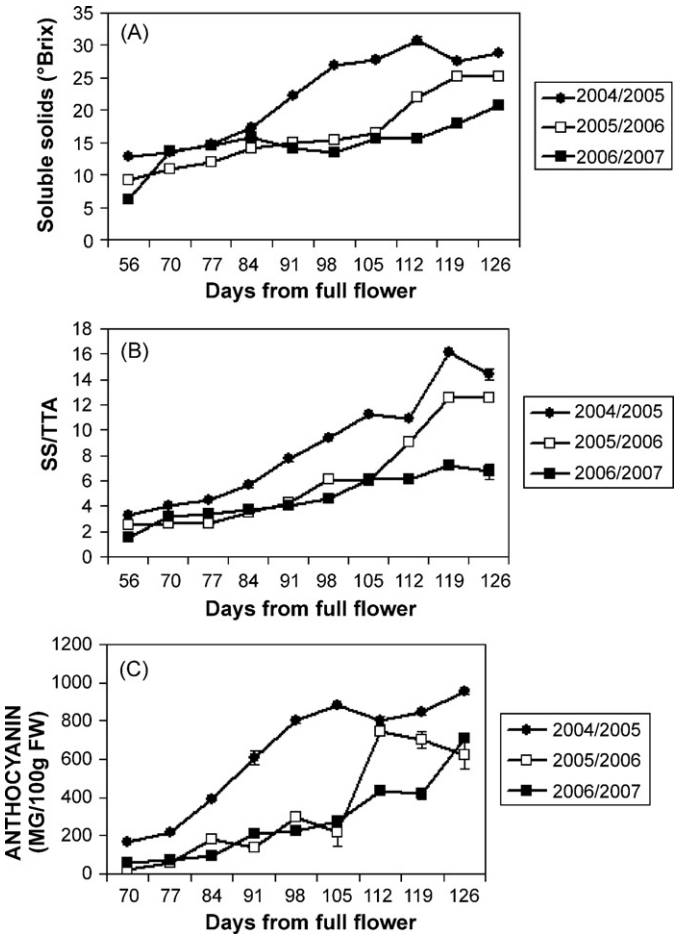

Fig. 5. *Berberis buxifolia* fruiting chemical properties. Soluble solids, soluble solid/total titratable acidity ratio, and anthocyanin content from day 56 to day 126 from full flower, during three growing seasons: 2004/2005, 2005/2006, and 2006/2007. Error bars represent ± S.E.

4. Discussion

4.1. Changes in fruit growth and chemical properties along the fruiting period

The growth rates of fruits vary greatly among species, cultural practices, and different fruits in the same crop. The period from anthesis to fruit ripening varies from about 3 weeks in strawberry to 60 weeks in orange, although in fruits of many species it is about 15 weeks (Kramer and Kozlowski, 1979). In the case of *B. buxifolia*, the fruiting period lasts about 18 weeks, while the maximum fruit fresh weight is attained in 12 weeks after the full flower phase.

It is well known that the kinetics of fruit growth exhibit two general patterns, the first being a simple sigmoid curve in which an exponential increase in size is followed by a deceleration of growth, all this resulting in a sigmoidal shape, a typical growth behavior of non-stone fruits. A second type of fruit growth consists of two successive sigmoidal growing periods separated by a lag phase of growth (Coombe, 1976; Gil-Albert Velarde, 2006), as was found for stone fruits, as well as for *Vitis* (Coombe and McCarthy, 2000) and *Ribes* (Wright, 1956; Fernqvist, 1961).

In *B. buxifolia*, the fresh and dry weight of the fruit nearly followed the double sigmoid growth pattern, and the same was observed when the growth was evaluated through the evolution of both the equatorial and polar fruit diameters, although in a less distinct sigmoid shape. It is possible to detect some growth variations caused by lag periods of fruit growth when seeds develop, and of course influences of environmental and/or cultural conditions cannot be discarded. In *B. buxifolia*, the first period of growth lasted from the full flower phase till approximately 42–56 days afterwards, similar to *Vitis* fruits (Kennedy, 2002), but longer than *Ribes* fruit growth (Toldam-Andersen and Hansen, 1997). It is during this first period of growth that the berry is formed and the seed embryos are produced. A rapid cell division occurs during the first few weeks, and by the end of the period, the total number of cells within the berry has been settled (Wright, 1956; Harris et al., 1968). The extent of cell division will at last be reflected on the definitive size of the berry. In the following lag or transition period,

the fleshy tissue grows slowly but the seeds continue to grow rapidly, as it was also cited for *Ribes* fruits (Toldam-Andersen and Hansen, 1997). The beginning of the second phase of *B. buxifolia* berry growth, i.e. the time when fruit ripening occur, is correlated with a number of changes in the plant metabolism, a process driven by energy derived from respiration. It has been previously reported that changes associated with fruit ripening include the loss of chlorophyll, which reveals other pigment loss (degradation), softening of the fruit flesh, development of odor and flavor, and a decrease in dry weight mainly due to respiration (Kramer and Kozlowski, 1979). Working in this case with *B. buxifolia* fruits, a 33% decrease in fruit firmness was found in the span extending from day 42 till day 84 after the full flower phase when the fruits attained maximum fresh weight, i.e. an average of 0.8% per day of ripening rate.

Almost 40 years of research have been devoted to the study of fruit softening mechanisms, most of it using tomato (*Solanum lycopersicum*) ripening as a model system. A decrease in fruit firmness typically is associated with dissolution of the middle lamella, resulting in a decrease in intercellular adhesion, depolymerization, and solubilization of hemicellulosic and pectic cell wall polysaccharides and, in some cases, wall swelling (Brummell and Harpster, 2001). Ripening is also accompanied by the increased expression of many cell wall degrading enzymes, including polysaccharide hydrolases, transglycosylases, lyases, and other wall loosening proteins, such as expansin (Harker et al., 1997; Rose et al., 2003; Brummell, 2006). Hence, models generally attribute fruit softening to degradation of polysaccharides in the primary wall and middle lamella (Rose et al., 2003; Brummell, 2006), although other factors such as cellular turgor and morphology had also been involved when considering fruit texture (Lin and Pitt, 1986; Shackel et al., 1991). According to this, it is expected that berry fruit softening in *B. buxifolia* could also be due to changes in the composition of cell walls of the fruit tissue. This was reported to be true for *Vitis* berry, in which ripening was particularly associated to pectin and xyloglucan depolymerization (Bisson, 2001).

All *B. buxifolia* fruit surfaces attain their final and characteristic purple color during the second phase of berry growth. The berries approximately double in size (in terms of dry fruit weight) during the span of fruit growth needed to reach the end of the second period of growth, presumably due to both cell division and enlargement of the fleshy tissue with the arrival of carbohydrates, nitrogen compounds, and other substances including minerals translocated from source tissues. A decrease in the fresh weight of fruits begins 84–98 days from the full flower phase, a fact that must be considered to determine the optimal time for harvest (Bisson, 2001). Thus, as a whole the observed changes in the fruit growth are associated with changes in fruit metabolism which include 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, and anthocyanin concentration changes. Soluble solids include carbohydrates, organic acids, proteins, lipids and several minerals, being sugars the main components of soluble solids in most of fruits (Wills et al., 1981). Soluble solids and acidity are relatively easy to assay, and are useful chemical traits to define the optimal time for harvest. Acidity can be evaluated as either pH or titratable acidity or both (Boulton et al., 1996). During *B. buxifolia* fruiting, an initial rapid phase of soluble solid accumulation can at first be ascribed to sucrose hydrolysis yielding glucose and fructose, and then, at some point of berry development and aging, an increase in soluble solid concentration can arise from fruit dehydration, as has been previously reported for *Vitis* (Bisson, 2001). The soluble solids found in ripened *B. buxifolia* fruits grown at Ushuaia averaging near 25.0°Brix were lower that the values cited for this species (33°Brix) grown in the south of Chile (Arribillaga García, 2001).

Anthocyanins are the major phenolic components of soft berry fruits, and their antioxidant activity was closely related to total phenolic content (Deighton et al., 2002). Anthocyanin concentration evolution during fruiting of *B. buxifolia* experienced a similar behavior to the soluble solids evolution, being its concentration maximal at the pick of soluble solids and when the total titratable acidity was at a minimum value. These secondary metabolites are produced during the plateau phase of the fruit growth curve. Lack of production during the early stages can be explained by carbon allocation mainly used to sustain biomass increase from primary metabolism when growth is very active. On the other hand, when growth ends, carbon is no longer needed in large quantities for primary metabolism and secondary compounds are more actively synthesized (Bourgaud et al., 2001). Anthocyanins abruptly increased when fresh fruit biomass reached the maximum value. The anthocyanin concentration found in *B. buxifolia* fruits at maturity was higher that the one reported for this species at the XI Region in Chile (Arribillaga García, 2001) and even higher than that corresponding to other reddish-purple berries, as *Ribes nigrum*, *Vaccinium* spp., *Rubus* spp. y *Fragaria* spp. (Burrows and Moore, 2002; Lister et al., 2002).

When analyzing the changes in fruit growth together with the evolution of the chemical properties along the fruiting period, it can be concluded that parameters such as fruit biomass and size attain the maxima values earlier (70–98 days from full flower phase) than chemical properties (105–112 days from full flower), and must be taken into account when defining optimum time of harvest according to the fruit utilization.

4.2. Changes in fruit growth and in chemical properties during different growing seasons

Generally speaking, the growth rate and composition of fruits greatly vary among seasons and environmental conditions (Biale, 1950; Boynton and Wilde, 1959; Kramer and Kozlowski, 1979; Predieri and Dris, 2005). Dramatic effects of environmental and cultural conditions on both sugar and anthocyanin contents have been reported for grape berries (Keller and Hrazdina, 1998; Bisson, 2001). The higher mean daily temperatures and earlier accumulation of growing degree days in 2004/2005 compared with the other growing seasons may, of course, be related with the higher fruit biomass and fruit diameters attained during the first and second month of fruit growth, as well as with the higher soluble solids, soluble solids/total titratable acidity ratio and anthocyanin content in the third and fourth month of fruit growth in the 2004/2005 growing season. It is already well established that temperature is a limiting factor of photosynthesis rates in plants, thus at higher temperatures, photoassimilates are produced at a higher rate, which in turn makes it possible to increase the translocation from leaves to different sinks, including the fruits (Madore and Lucas, 1995; Zamski and Schaffer, 1996). Among small fruit berries, it was found for *Ribes*, that fruit growth, is also correlated with climate conditions (Toldam-Andersen and Hansen, 1993).

The studied parameters of fruit growth and chemical properties of *B. buxifolia* showed significant changes along its fruiting period and among the studied growing seasons, so they could be considered as good markers of the fruiting phases in this wild small fruit species. However, it is necessary to define other physiological and chemical markers such as fruit respiration and antioxidant activity in order to design adequate tools for defining the optimal time of harvest according to fruit utilization (fresh fruit, industrial uses, etc.), as well as to keep its nutraceutical properties.

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