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Fruit quality of black currants produced in Tierra del Fuego, Argentina

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Abstract

Small fruits are of great interest due to the increasing world demand, and particularly, attention is focused on many species which are now considered for their nutraceutical properties. This work studied the fruit growth and composition along the ripening period (February) and among different years of three varieties of *Ribes nigrum* L. (Rosenthal, Titania and Silvergieters), growing in Ushuaia, Tierra del Fuego, Argentina. There were not significant differences in physical and chemical characteristics among the tested varieties, except for anthocyanin content, Silvergieters being the variety with the highest anthocyanin content (67.8 mg/100 g fresh fruit weight) and February 15th when the fruits attained maximum content (87.7 mg/100 g fresh fruit weight). The higher temperatures in 2004/2005 compared with the other growing seasons may be related with the higher fruit weight and diameter, as well as with the higher soluble solids and soluble solids/total titratable acidity ratio. It is expected that the information obtained throughout this study could be of value in defining the optimal time for harvesting of *R. nigrum* fruits according to their future use, while contributing to the knowledge of their nutraceutical properties through the quantitative content of soluble solids, total titratable acidity, and anthocyanins.

Key words: *Ribes nigrum*, small fruits, South Patagonia, ripening, anthocyanin.

Introduction

The industrial demand for berries worldwide is well known. Particularly, attention is focused on many of the small fruits which are now not only considered as a source of minerals and vitamins, but also considered for their nutraceutical properties as functional foods, i.e. foods containing some specific metabolites which give additional benefits for health [1, 2]. Soft fruits of *Ribes*, *Rubus* and *Vaccinium* species are an excellent source of natural products as pigments [1, 3], with antioxidant properties [4]. *Ribes* species are cultivated for several uses, such as for preserves, juice and teas[5], for the extraction of anthocyanins from *R. nigrum* fruits with considerable health benefits [6] and the extraction of oils in highly unsaturated fatty acids from the seeds [7].

The berry fruit species, in particular belonging to the genera *Ribes* and *Rubus*, are important for diversification of production in mountainous and marginal areas such as Southern Apennines [5, 8]. The fruit, both for food and non-food (pharmaceutical, cosmetic industries) use, is a source of revenue for people living in marginal areas. In Argentinean Patagonia, an increasing interest has been shown to the intensive agriculture over the past years [9, 10]. This is due to its diversity in soils and climates and, mainly, to the possibility of producing out of season, with regard to the north hemisphere. Likewise, in Argentina, berries are currently of increasing importance for the local industry as for the fresh market. Andean Patagonia is the most important zone for producing *Rubus* and *Ribes* berries in Argentina, particularly the Andean region of the 42nd parallel. The *Ribes* culture, however, is still incipient, with a cultivated area of about 10 ha in the cited region. Nevertheless, cold hardiness and early ripening make *Ribes* a viable alternative, in such regions as Tierra del Fuego, where winters are too cold or growing seasons too short and cool for the culture of grapes, other berries or tree fruit crops [11].

Hence, the aim of this work was to study the fruit growth and composition along the ripening period and among different years of three varieties of *Ribes nigrum* L. (Rosenthal, Titania and Silvergieters), growing in Ushuaia, Tierra del Fuego, Argentina. It is expected that the information obtained throughout this study could be of value in defining the optimal time for harvesting *Ribes nigrum* fruits according to their future use, while contributing to the knowledge of their nutraceutical properties through the quantitative content of soluble solids, total titratable acidity and anthocyanins.

Materials and Methods

Geographic and climatic description: The studied area is located near Ushuaia city, 54º48´SL, 68º19´WL (Tierra del Fuego, Argentina). Climatic data on maximal, minimal and mean air daily temperatures (ºC); mean ambient relative humidity (%) and accumulated rainfall (mm) were recorded by the Meteorological Station at Centro Austral de Investigaciones Científicas (www.cadic.gov.ar, SIAG) from October to March (growing season period) for the years 2004/05, 2005/06 and 2006/07.

Plant material, sampling and measurements: Plants of *Ribes nigrum* L. varieties Rosenthal, Titania and Silvergieters (n = 20 of each variety), of eight years old at the beginning of the experiment, growing at the experimental field of the Centro Austral de Investigaciones Científicas were used for the experiment. These varieties were selected because they are most commonly grown

in the Argentine Patagonian region. The irrigation, fertilization and weed control were as was described previously for *R. rubrum* varieties [12]. Fruit samples (200 g) were collected from all the orientations of the shrubs at the beginning and middle February (when the fruits ripen), during the years 2005, 2006 and 2007. The following variables were recorded in each randomized fruit (n = 20) from the samples: fresh fruit weight, dry fruit weight (using a drying oven at 40ºC), dry fruit weight concentration, equatorial and polar fruit diameters (using a digital caliper Mitutoyo Model 500-196 (150 mm x 6" – 0.01 mm x 0.0005"), fruit firmness, using a digital penetrometer Wagner Instruments (USA) Model FDI 2, 0.001 a 1 kgf, with tips of 1 mm diameter.

Soluble solids were determined in fruit juice using an ATAGO N1-α refractometer with 0 to 32°Brix measurement range with 0.2 increments, with no temperature compensation. Total titratable acidity was measured by manual titration equipment and a peachimeter, using a 0.1 N NaOH solution. 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 [13]. Samples (5 g) of initially frozen fruits were extracted during 24 h in 50 ml 0.1% HCl-MeOH solution at 4°C. Then, aliquots were diluted from 1:5 to 1:10 with either a 0.025 M KCl (pH 1) or a 0.4 M sodium acetate (pH 4.5) buffers. Absorbance measurements were made at 510 and 700 nm in a Shimadzu 1203 UV-Visible spectrophotometer. Anthocyanin fruit tissue content was determined on a cyanidin 3-glucoside molar extinction coefficient of 26,900 and a molecular weight of 449.2. Resultant values were expressed in terms of mg of anthocyanin/ 100 g of fresh-frozen fruit. Anthocyanin (mg /100 g fruits) = (A x molecular weight x dilution factor x initial volume/ε x sample weight) x 100, where A (absorbance) = $(A_{510nm} - A_{700nm})_{pH\ 1.0}$ - $(A_{510nm} - A_{700nm})_{pH\ 4.5}$

Statistical analysis: Data were subjected to a multifactor analysis of variance, where means were separated through the Tukey multiple range test at $p \leq 0.05$.

Results

Climatic description: Mean daily temperature was higher (9.0°C) during 2004/05 than in 2005/06 and 2006/07 growing seasons (8.3 and 8.5°C, respectively). The greatest differences in mean daily temperatures (near 3.0°C) were found in November and February among the studied growing seasons (Fig. 1A). Maximal daily temperatures were higher (13.5°C) during 2004/05 than in the following growing seasons (12.8 and 12.9°C, respectively), as well as minimal daily temperatures (4.6, 4.4 and 4.4°C) for the 2004/05, 2005/06 and 2006/07 growing seasons, respectively. Mean ambient relative humidity was 74.9, 90.6 and 70.2% for the 2004/05, 2005/06 and 2006/07 growing seasons, respectively. Total rainfall reached the maximum value (324.4 mm) in 2005/06, being of 295.6 and 300.0 mm in 2004/05 and 2006/07 growing seasons, respectively (Fig. 1B). The maximum rainfall occurred in December for the 2004/05 growing season, while in January for the 2005/06 growing season and later (February and March) for the 2006/07 growing season.

Fruit growth and composition of R. nigrum varieties during the ripening period and among different years: The fresh and dry fruit weight only varied significantly among the years (Table 1). Rosenthal variety presented the highest fresh fruit weight (828.7

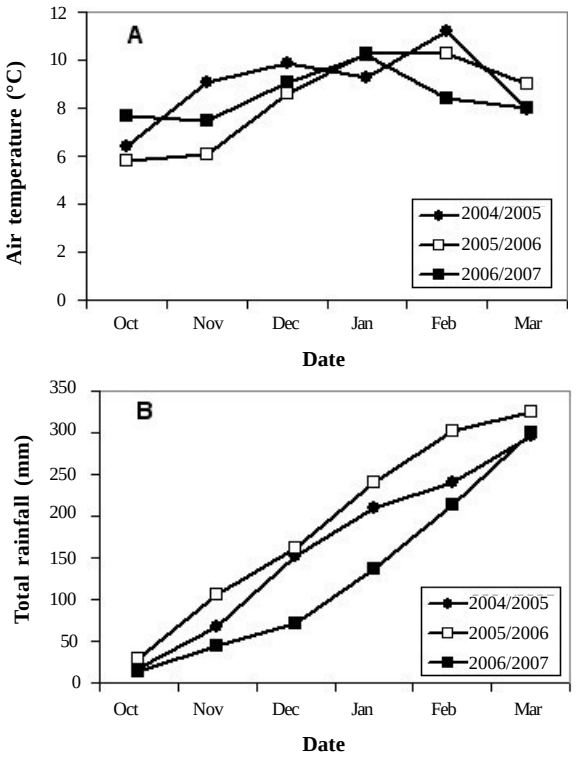

Figure 1. Mean air daily temperatures and accumulated rainfall recorded from October to March for the 2004/05, 2005/06 and 2006/07 seasons.

mg), this variable being maximum at February 1st. The highest fresh fruit weight (1011.3 mg) was reached in 2005 when compared with the other years (700.5 and 713.5 mg for the years 2006 and 2007, respectively). Silvergieters variety presented the highest dry fruit weight (172.6 mg), while this parameter was higher (219.0 mg) in 2005 than in the years 2006 and 2007 (141.3 and 142.2 mg, respectively). The dry fruit weight concentration significantly varied along the ripening period and year (Table 1), being maximal (21.6%) on February 15th and in 2005 (21.8%). The equatorial fruit diameter was affected only by the year, presenting in 2005 its maximum values (11.9 mm). The polar fruit diameter did not present statistical differences in the studied factors, being close to 10.1-10.6 mm. Firmness did not vary significantly neither with the varieties nor with the ripening period, reaching values between 0.15 and 0.19 kgf (data not shown).

Significant interactions were found in the described variables, particularly among variety and ripening and ripening and year factors, mainly due to different value increments between the mentioned main factors (Table 1) and differences between combinations (Fig. 2), e.g. fresh and dry fruit weight in Silvergieters variety had different behaviors in 2006 in comparison with the years 2005 and 2007 (Fig. 2E-F).

The soluble solids significantly varied during the ripening period and among years (Table 2). This variable was maximal (15.7°Brix) on February 15th. The soluble solids were significantly higher (16.4°Brix) in 2005 than in the other years (13.9 and 13.5°Brix for the years 2006 and 2007, respectively). The total titratable acidity did not vary significantly among the studied factors (Table 2), reaching values of 2.9 to 3.0%. However, the soluble solids/total titratable acidity ratio was affected by the ripening and year (Table 2). This variable was maximal on February 15th (5.6) and in 2005

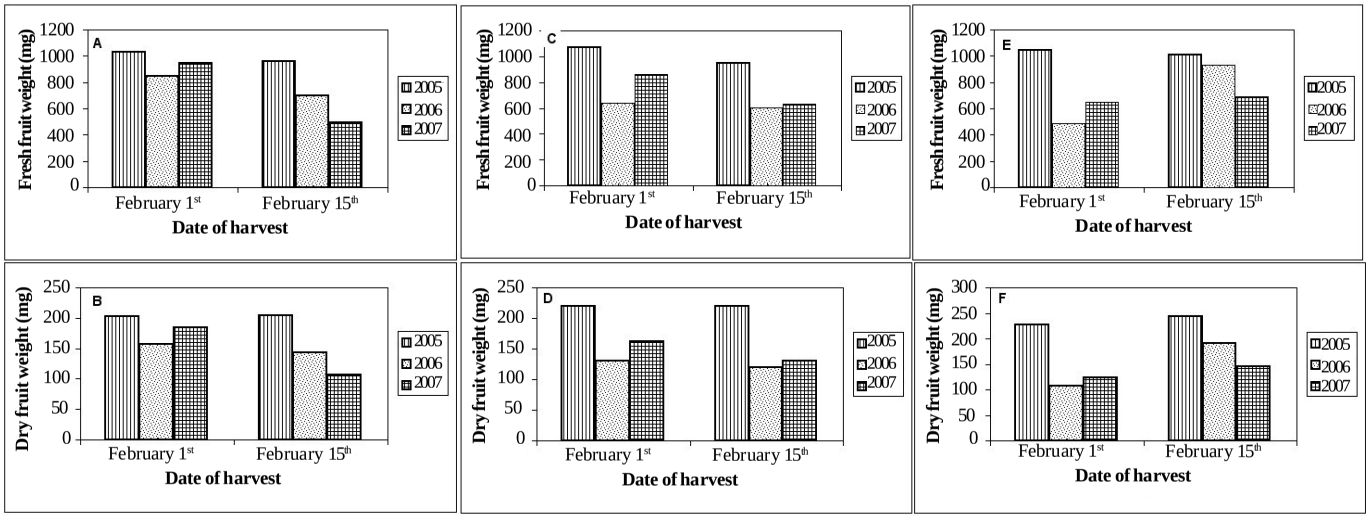

Figure 2. Fresh fruit weight and dry fruit weight analyzed in *R. nigrum* fruits (A-B Rosenthal; C-D Titania and E-F Silvergieters varieties), during February of 2005, 2006 and 2007 years.

Table 1. Means values of ANOVA analyzing fruit growth of *Ribes nigrum* considering variety, date during the ripening period and year as main factors, and fresh fruit weight (mg) (FFW), dry fruit weight (mg) (DFW), dry fruit weight concentration (%) (DFWC), equatorial fruit diameter (mm) (EFD) and polar fruit diameter (mm) (PFD) as dependent variables (n=20).

Main effects	FFW	DFW	DFWC	EFD	PFD
A = Variety					
Rosenthal	828.75	166.52	20.50	11.33	10.49
Titania	793.78	163.47	20.47	11.21	10.35
Silvergieters	802.82	172.60	21.50	11.27	10.41
F(p)	0.25(0.780)	0.41(0.662)	2.50(0.085)	0.10(0.905)	0.16(0.854)
B = Date during the ripening period					
Feb 1st	842.57	167.83	20.05b	11.39	10.52
Feb 15th	774.33	167.22	21.60a	11.15	10.32
F(p)	2.63(0.106)	0.01(0.941)	13.16(0.000)	1.34(0.249)	1.01(0.315)
C = Year					
2005	1011.30a	219.03a	21.85a	11.93a	10.64
2006	700.52b	141.31b	20.11b	10.99b	10.50
2007	713.53b	142.25b	20.51b	10.89b	10.11
F(p)	23.30(0.000)	38.03(0.000)	6.08(0.002)	10.01(0.000)	2.46(0.088)
Interactions F(p)					
A x B	7.19(0.001)	6.55(0.001)	0.41(0.661)	7.16(0.001)	7.70(0.000)
A x C	0.81(0.518)	1.34(0.255)	1.81(0.129)	0.59(0.673)	1.71(0.150)
B x C	4.15(0.017)	2.92(0.056)	3.47(0.033)	3.12(0.046)	2.44(0.090)
A x B x C	1.55(0.189)	1.22(0.304)	0.60(0.660)	1.37(0.247)	1.10(0.360)

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.

Table 2. Means values of ANOVA analyzing fruit composition of *Ribes nigrum* considering variety, date during the ripening period and year as main factors, and soluble solids (º Brix) (SS), total titratable acidity (%) (TTA), soluble solids/ total titratable acidity relation (RATIO), initial pH (pH), and anthocyanin content (mg anthocyanin/100 g fresh fruit) (ANTH) as dependent variables (n = 6).

Main effects	SS	TTA	RATIO	PH	ANTH
A = Variety					
Rosenthal	14.71	2.97	5.09	3.18	57.73b
Titania	14.65	2.96	5.09	3.17	60.68ab
Silvergieters	14.38	2.90	5.11	3.17	67.79a
F(p)	2.41(0.104)	0.08(0.921)	0.00(0.995)	1.18(0.330)	5.75(0.005)
B = Date during the ripening period					
Feb 1st	13.47b	3.01	4.64b	3.16b	36.47b
Feb 15th	15.68a	2.88	5.56a	3.19a	87.66a
F(p)	281.49(0.000)	0.61(0.446)	12.33(0.002)	17.90(0.000)	393.4(0.000)
C = Year					
2005	16.36a	2.86	5.74a	3.14b	
2006	13.87b	3.00	4.79b	3.10c	73.54a
2007	13.51c	2.97	4.76b	3.28a	50.60b
F(p)	226.97(0.000)	0.25(0.780)	6.03(0.009)	232.69(0.000)	78.99(0.000)
Interactions F(p)					
A x B	1.84(0.172)	0.30(0.741)	0.21(0.808)	20.67(0.000)	8.55(0.000)
A x C	3.98(0.008)	1.16(0.360)	0.68(0.613)	32.15((0.000)	4.03(0.023)
B x C	24.13(0.000)	2.97(0.076)	5.75(0.011)	0.15(0.863)	49.50(0.000)
A x B x C	1.13(0.358)	1.48(0.250)	1.02(0.424)	6.64(0.001)	21.94(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.

(5.7). The initial pH significantly varied along the ripening period and among the years (Table 2). This variable was maximal (3.2) on February 15th and presented the highest values (3.3) in the year 2007.

Anthocyanin content varied between varieties, during the ripening period and years (Table 2). This variable was higher in Silvergieters variety (67.8 mg anthocyanin/100 g fresh fruit weight) than in Titania (60.7 mg anthocyanin/100 g fresh fruit) and Rosenthal (57.7 mg anthocyanin/100 g fresh fruit) varieties. Also, it was higher (87.7 mg/100 g fresh fruit) on February 15th than earlier (36.5 mg/100 g fresh fruit). Anthocyanin content was higher in 2006 than in 2007 (73.5 and 50.6 mg/100 g fresh fruit, respectively).

Significant interactions were found in the described variables among several of the studied factors, mainly due to different value increments between the main factors (Table 2) and differences between combinations (Fig. 3), e.g. total titratable acidity and soluble solids/total titratable acidity ratio in Silvergieters variety had different behaviors along the studied period (Fig. 3J-K), as well as anthocyanin content (Fig. 3L).

Discussion

Fruit growth and composition of R. nigrum varieties during the ripening period: The fruit ripening phase is characterized by a variation in physical fruit characteristics and 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 pigments loss (degradation), softening of the fruit flesh, development of odor and flavor, and a decrease in dry weight mainly due to respiration [14]. 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 [15]. In *R. nigrum* fruits, only anthocyanin content presented significant differences among the tested varieties, Silvergieters being the variety with the higher values. Soluble solids, ratio, pH and anthocyanin content increased significantly along the ripening period, these variables being maximal on February 15th. The soluble solids found in ripen *R. nigrum* fruits grown at Ushuaia along the studied period averaging close to 13.5 to 15.6°Brix were a little higher than those for other *Ribes* species, as *R. grossularia* growing in Norway[16]. However, the ratio values were higher than those found for *R. nigrum* varieties Tenah and Ben Nevis [17]. Anthocyanins are the major phenolic components of soft berry fruits [4], and their concentration in *R. nigrum* fruits at maturity were lower than for *R. nigrum* varieties Titan and Öjebyn [18] and those cited by Lister *et al.* [6], higher than for *R. rubrum* var Red Dutch [18] and comparable to other reddish-purple berries as *Rubus* and *Fragaria* spp.[6, 19].

Fruit growth and composition of R. nigrum varieties during the years: The growth rate and composition of fruits greatly vary among seasons and environmental conditions [14, 20, 21]. Dramatic effects of environmental and cultural conditions on both sugar and anthocyanin contents have been reported for grape berries [22, 23]. The higher mean daily temperatures in 2004/05 compared with the other growing seasons may of course be related with the higher *R. nigrum* fruit weight and fruit diameters, as well as with the higher soluble solids and soluble solids/total titratable acidity ratio in the fruits in the year 2005. The different behaviour in fresh and dry fruit weight in Silvergieters variety shown between the beginning and middle February in 2006, in comparison with 2005 and 2007 (Fig. 2E-F), could be due to the lower temperatures during the beginning of the fruiting process. The higher mean daily temperatures in February 2006 (10.3ºC) compared with February 2007 (8.4ºC) could be related with the higher *R. nigrum* anthocyanin content in 2006 than in 2007. It is already well established that temperature is a limiting factor of photosynthesis rates in plants, thus at those higher temperatures, photoassimilates will be produced at a higher rate, which in turn will make possible to increase the translocation from leaves to different sinks, including the fruits [24, 25]. Among small fruit berries, it was found for *Ribes nigrum* that fruit growth and anthocyanin content is also correlated with climate conditions [26, 27].

Conclusions

The results obtained in this work highlight the importance of considering both physical and chemical variables to determine the optimal time of harvest due to the particular behaviour of each of them. Indeed, these results are the first antecedents on the knowledge of *Ribes nigrum* fruit quality at Ushuaia, Tierra del Fuego. Further studies on secondary metabolite content in correlation with antioxidant properties will contribute to the knowledge of nutraceutical value of these *R. nigrum* varieties.

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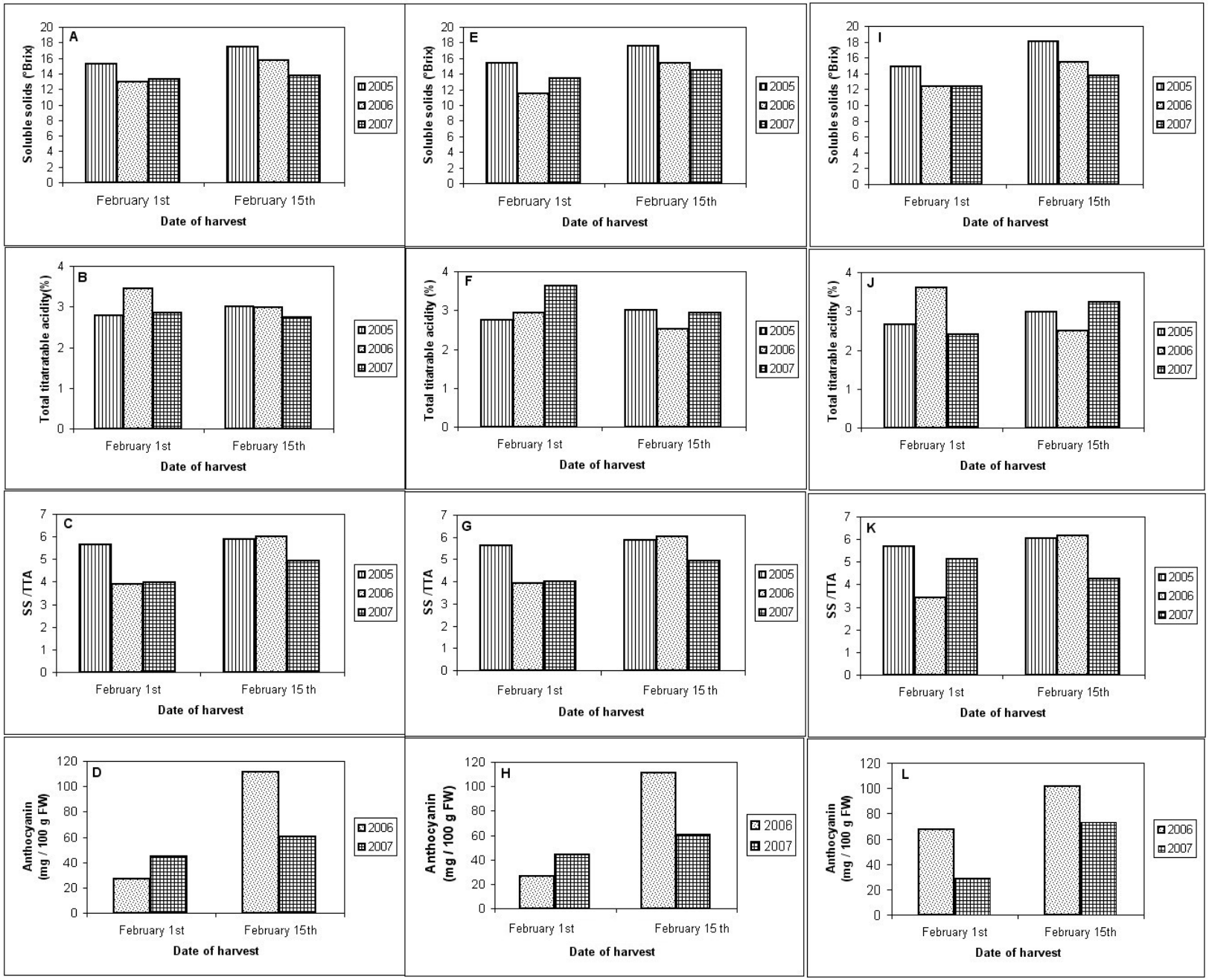

Figure 3. Soluble solids, total titratable acidity, soluble solid/total titratable acidity ratio and anthocyanin content analyzed in *R. nigrum* fruits (A-B-C-D Rosenthal; E-F-G-H Titania and I-J-K-L: Silvergieters varieties), during February of 2005, 2006 and 2007 years.