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Fruit quality parameters of some southern highbush blueberries (*Vaccinium xcorymbosum* L.) grown in Andalusia (Spain)

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Abstract

Physical and chemical parameters of fresh berries from three southern highbush ('O'Neal', 'Sharpblue' and 'Misty') blueberry cultivars grown in Huelva (Southwestern Spain) under two production systems were measured and evaluated. ANOVA applied to data yielded significant differences between production systems for mean fruit size and mean fruit fresh weight ($P<0.05$), although main effects on physical and chemical characteristics of fruit were due to cultivar. All three cultivars showed significantly different means ($P<0.05$) for fruit fresh weight, and all chemical characteristics. Stepwise discriminant analysis (SDA) for classification and identification of the cultivars based on physico-chemical properties of samples of fruits was performed. The model obtained gave high percentages of correct classification and prediction (81.1% and 78.4%, respectively). The variables with higher discriminating power were fruit titratable acidity, fruit size and fruit sugar content.

Additional key words: discriminant analysis, 'Misty', 'O'Neal', production system, 'Sharpblue'.

Resumen

Parámetros de calidad de frutos de algunos cultivares de arándano americano (*Vaccinium xcorymbosum* L.) en Andalucía

Se midieron parámetros físicos y químicos de frutos frescos de arándano americano, correspondientes a tres cultivares de bajos requerimientos en horas frío ('O'Neal', 'Sharpblue' y 'Misty') cultivados en Huelva (suroeste de España) bajo dos sistemas de producción. Los datos se sometieron al análisis de varianza (ANOVA) de 2 factores. No se encontraron diferencias significativas ($P>0,05$) en la firmeza, contenido en azúcar, pH o acidez titulable en los dos sistemas productivos, mientras que los tres cultivares ensayados arrojaron medias diferentes ($P<0,05$) para el peso fresco del fruto y todos los parámetros químicos controlados. Se aplicó un análisis discriminante por pasos para la clasificación y diferenciación de los cultivares en función de las variables físico-químicas de las muestras de frutos, obteniéndose altos porcentajes de clasificación correcta y predicción (81,1% y 74,8% respectivamente). Las variables con mayor poder discriminante fueron la acidez titulable, el tamaño y el contenido en azúcar del fruto.

Palabras clave adicionales: análisis discriminante, 'Misty', 'O'Neal', producción, 'Sharpblue'.

Introduction

About 10% of the world blueberry (*Vaccinium* spp.) area was in Europe in 2003, and there were an estimated 250 ha in Spain and Portugal, accounting for the 7.2% of the cultivated total surface (Strik, 2005). Cultivated blueberries were introduced into western Andalusia from North America in the early 1990s, where commercial crops have only been a regular feature since 1995. Today, low-chill highbush blueberry plantings and production have been increasing in the southwestern region of Spain due to the good climatic and soil characteristics and high market profitability, and blueberries are a well established berry crop along with strawberries (Barrau *et al*., 2004, 2006; Strik, 2005). Two main production systems exist in Andalusia, open

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Abbreviations used: SDA (stepwise discriminant analysis), SE (standar error).

air cultivation, and protected cultivation, with the use of plastic tunnels and shade cloths (Sobey, 2004; Renquist and Strik, 2005); this later system has become the most widely used for blueberries in our region. Little information is still available on its effects over several aspects of the culture such as variations in plant phenology, fruit quality and phytosanitary status. In fact few data on the fruit quality of the major American cultivars growing in our local conditions are known. With the beginning of commercial production in Andalusia it became clear that accelerate ripeness protecting fruit quality and characteristics would be very important. There has become an increased awareness of the need to harvest fruit at a satisfactory state of ripeness and to have the best fruit possible from the cultivar concerned. This paper reports and evaluates data on blueberry fruit quality in Huelva, trying to determine differences between production systems, and significant physico-chemical variables that could contribute to the differentiation of fruit samples from the different cultivars assayed.

Material and methods

Plant material and experimental plots

This research was conducted during two consecutive fruit seasons (2004-2005) using 'O'Neal', 'Sharpblue' and 'Misty' blueberry plants grown at the Experimental Research Station of 'El Cebollar' (IFAPA, Moguer, Huelva). Southern highbush cultivars were selected based on their low chilling requirements, high quality and widespread using in plantings both in the world and in Andalusia (Strik, 2005). 'Sharpblue' is the leading early variety in low-chill areas throughout the world and is considered a standard cultivar to which others are compared (Zee *et al*., 2006). Plots were planted in 2003, using 2-yr old potted plants from a commercial nursery (Viveros Huelva, S.A., Moguer, Huelva, Spain), in a randomized block design with four replicates of each cultivar. Thirty-two plants of each cultivar were randomly assigned to experimental plots that supported different management systems: open air (non-protected system), or a high hoop plastic tunnel (protected system). Plastic covers were installed each year in late autumn (November), and removed in late spring (May/June), when they were replaced by shade cloths in order to provide protection for the higher summer temperatures of this region. In late September, shade cloths were removed, and blueberry plants were let at open air until the plastic covers were installed for the next crop season.

Fruit sampling

In 2004 and 2005, during each fruit season ripeness was determined by colour, and harvested by hand in intervals of 7 to 10 days. Sixteen harvest dates (weeks) were considered in this study. At each picking date, 150 g samples of fruits were placed directly into plastic clamshell containers (14 x 9.5 x 5 cm, 250 g, PET, Autobar Spain, Barcelona, Spain), and transported in cold into a portable icebox to laboratory. Subsamples of 20 berries were used to measure fresh weight, fruit size (equatorial diameter), and firmness (skin toughness). Fruit juice (see below) was prepared for analysis of the following chemical variables: sugar content (°Brix), titratable acidity (g citric acid/100 mL) and pH. All measurements and analyses were made 24-48 h after harvest and replicated twice.

Physico-chemical characteristics of fruits

Fresh weight was measured using an electronic digital balance (Mettler AE240) to 0.01 g sensitivity. For measures of equatorial width of fruits an electronic digital calliper was used with 0.01 mm resolution. Firmness was estimated by means of puncture tests done using a TR penetrometer (model FDP 500, Effegi, Italy) fitted with a 2.0 mm diameter probe. For each fruit tested two punches were done in the equatorial part of the berry while the berry stood on a flat, horizontal surface, the mean penetration force (g mm^{-2}) of the two punches was used as data for further analysis. Measurements were made by the same operator in order to minimize possible inaccuracies (Harker *et al*., 1996).

Samples of fruits were hand grinded and the pulp obtained centrifuged at 4000 rpm for 15 min; the clear upper phase obtained was used for chemical determinations. Total soluble solids concentration was determined by placing 1 mL of blueberry juice on a portable refractometer (Eclipse, Bellingham and Stanley Ltd., UK), and expressed as °Brix. Titratable acidity was determined using a standard titration with 0.1 N sodium hydroxide, and quantified as citric acid equivalents (g citric acid/100 mL), pH was determined using a Crimson pH-

meter basic 20 (Alella, Spain). The sugar/acid ratio, which contributes toward giving fruits their characteristic flavour, was also calculated.

Statistical analysis

Data were analysed by two-way ANOVA procedure (SPSS Inc. Win version 12.0) to test differences between management systems and cultivars, posthoc Scheffé tests were applied for multiple comparison. Stepwise discriminant analysis (SDA) was also carried out using the 'forward' procedure, which begins with no variables in the model and adds the variables with the greatest discriminating power. The classification functions corresponding to each blueberry cultivar were calculated. The selection of variables was performed using the F statistics. Firmness, size, pH, sugar content and titratable acidity were selected as initial variables for discrimination. Fruit fresh weight and sugar/acidity ratio were not used in analysis because of their linear relation with fruit size, sugar and acidity values.

Results

Fruit characteristics

Significant differences ($P<0.05$) between the three cultivars for fruit fresh weight, pH, sugar content, titrable acidity, and sugar/acidity ratio were found. No significant differences for skin firmness neither fruit size were observed. 'Misty' fruits were significantly lighter than those of 'O'Neal' and 'Sharpblue'. Great puncture force was needed to penetrate the fruit skin of this cultivar. Mean berry size varied between 1.4 cm in 'Sharpblue' and 1.1 cm in 'Misty'. Mean fruit size did not statistically differ among cultivars, but plants inside plastic tunnels yielded smaller fruits than those placed at open air (Table 1, Fig. 1).

The comparison between the two production systems showed that there were significant differences in fruit size and fresh weight; both variables were higher in plants growing at open air. Firmness was slightly higher when plants were grown under plastic tunnels, except for 'Sharpblue' for which diminished firmness were detected, although no significant differences were observed (Tables 1 and 2; Fig. 1).

ANOVA yielded significant differences in chemicals characteristics (Table 1); pH values oscillated between 3.2 and 2.8. Fruit sugar content varied between 12.7 and 9.8, 'Misty' was the cultivar with sweeter fruits. The highest titratable acidity value was also obtained for 'Misty' varying between 9.0 and 5.4. Sugar/acid ratio was higher in 'O'Neal', with mean values oscillating between 1.9 and 1.4.

The comparison between plants growing at open air and plants growing in plastic tunnels shows that there were no significant effect on chemical properties of fruits (Table 2). In spite of this, 'O'Neal' showed higher pH values and lower sugar content and titratable acidity than the other cultivars in open air plots as well in tunnel/shade cloth plots (Fig. 1).

Cultivar x production interactions were not significant for any of the seven variables analyzed (Table 1).

Table 1. Two-way ANOVA F-test statistics for differences caused by cultivar and production system on blueberry fruit quality parameters

Fruit parameters	Main effects of cultivar	Main effects of management	Cultivar x management interaction
Firmness (g mm^{-2})	$F_{2,47}$ =0.09, P=0.918	$F_{1,47}$ =0.14, P=0.711	$F_{2,47}$ =1.11, P=0.341
Fresh weight (g)	$F_{2,57}$ =4.04, P=0.024	$F_{1,57}$ =6.64, P=0.013	$F_{2,57}$ =0.78, P=0.463
Size (cm)	$F_{2,57}$ =3.52, P=0.370	$F_{1,57}$ =5.67, P=0.021	$F_{2,57}$ =0.83, P=0.443
pH- value	$F_{2,54}$ =5.30, P=0.008	$F_{1,57}$ =0.26, P=0.612	$F_{2,57}$ =0.52, P=0.599
Sugar content (°Brix)	$F_{2,54}$ =30.00, P<0.0001	$F_{1,54}$ =0.51, P=0.480	$F_{2,57}$ =1.93, P=0.156
Titratable acidity (g citric/100 mL)	$F_{2,50}$ =27.80, P<0.0001	$F_{1,50}$ =0.25, P=0.618	$F_{2,50}$ =0.68, P=0.512
Sugar/acidity	$F_{2,50}$ =6.80, P=0.003	$F_{1,50}$ =0.19, P=0.668	$F_{2,57}$ =0.29, P=0.753

Table 2. Effect of cultivar on quality parameters (means±SE) of blueberry fruits. Means in the same column followed by the same letter are not significantly different by Scheffé test (P>0.05)

Cultivar	Fresh weight (g)	Size (cm)	Firmness (g mm^{-2})	pH-value	Sugar content (°Brix)	Titratable acidity (g citric/100 mL)	Sugar / acid ratio
'O'Neal' (n=29)	1.2±0.1 a	1.3±0.0 a	126.4±4.5 a	3.2±0.1 a	9.8±0.4 b	5.4±0.4 b	1.9±0.1 a
'Sharpblue' (n=28)	1.4±0.1 a	1.4±0.0 a	122.2±4.8 a	2.8±0.1 b	12.4±0.4 a	9.4±0.4 a	1.4±0.1 b
'Misty' (n=19)	0.9±0.1 b	1.1±0.0 a	127.9±6.2 a	2.8±0.1 b	12.7±0.5 a	9.7±0.6 a	1.4±0.2 b

Discriminant analysis of blueberry fruits by cultivar

The discriminant analysis was performed by steps with five of the variables measured. The best model selected three variables: fruit titratable acidity, fruit size, and fruit sugar content. The greatest percentage of correctly classified cases corresponds to the cultivar 'Sharpblue' with almost 93.3%. 'O'Neal' and 'Misty' fruits were well discriminated. 'Misty' accumulated the greater number of incorrect classifications. The graphic representation of fruits samples defined by the first two canonical functions (Fig. 2) shows a good separation of 'O'Neal' fruits on function 1; samples of 'O'Neal' are located in the negative part of the axis; whereas 'Sharpblue' and 'Misty' samples were located in the positive one. 'Sharpblue' and 'Misty' samples were slightly separated by function 2 axis, with 'Misty' fruit samples located mainly in the negative part (Tables 3 and 4). Taking into account that the distance between centroids is proportional to the similarity between groups, fruits samples of 'O'Neal' were the most specific, while fruits samples from 'Sharpblue' and 'Misty' were similar each other. The greatest percentage of correctly classified cases corresponded to the 'Sharpblue' fruits (93.3%) followed by those of 'O'Neal' (85.7%). With 'Misty' fruits there were a greater number of incorrect classifications (Table 4). The evaluation of the model was done by cross-validation, obtaining a global percentage of correct classification of 81.1% and a global prediction of 74.8%. These results can be considered satisfactory and acceptable (Fig. 2).

Discussion

The time during the season that fruit ripens has a large influence on its market value (Sobey, 2004). Plastic tunnels and summer shading nets are now viewed as good tools to manipulate the time of ripening obtaining off-season production of great economic value. Our region permits us to obtain earlier yields, low in quantity, but high in market value; however inconsistent yields from year to year when grown in protected systems have been reported elsewhere (Barrau *et al*., 2004; Forney, 2005; Renquist and Strik, 2005). In this study, fruit quality cha-

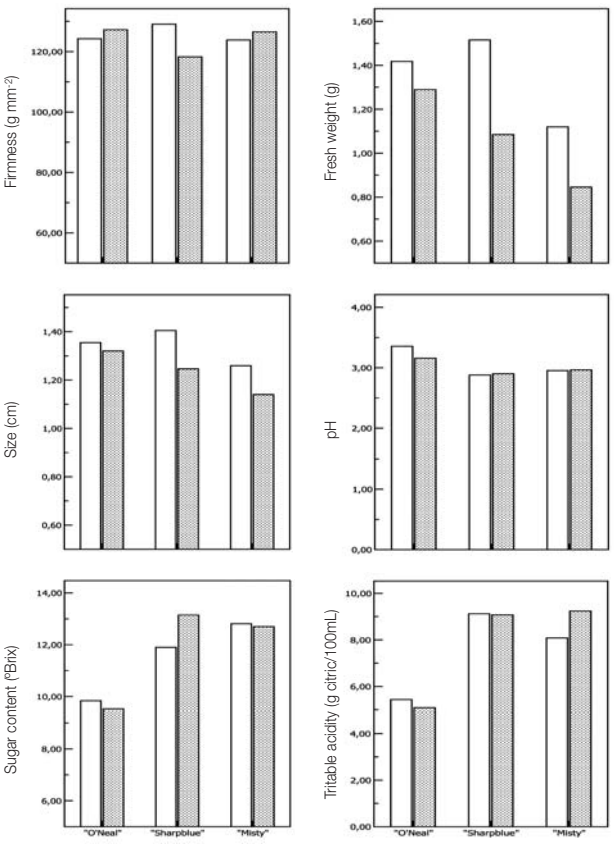

Figure 1. Quality parameters of three southern highbush cultivars grown in Andalusia into two production systems. Data presented as mean values. Clear bar: open air plots; shady bars: tunnel/shade cloth plots.

Table 3. Structure matrix

Variables	Function	
	1	2
Titratable acidity (g citric/100 mL)	0.741	0.034
Sugar content (°Brix)	0.523	-0.082
pH[a]	-0.256	-0.188
Size (cm)	-0.023	0.931
Firmness (g mm^{-2})[a]	-0.095	-0.105

[a] Variable not used in the analysis.

racteristics analyzed were not much affected by the use of a protected system production.

Usually, blueberry plants do not reach typical fruit size and production until 7-8 years. In Huelva, a common task practiced by growers with young plants in blueberry orchards, is to remove a certain amount of flowers in order to obtain fruits of greater size. As this task was not done during this study, the abundant fruit load present in some plants may be the cause by which fruit size values are under the means published elsewhere, as was noted by Zee *et al.* (2006). Fruit size and fresh weight mean values for the cultivars assayed were all over premium market fruit size (>0.75 g).

The decrease in these parameters observed when fruit samples are compared by production system may reflect pollination problems for plant growing in protected plots (Lang and Danka, 1991). Plastic tunnels accelerate plant phenology, but this may cause a temporal asynchrony between peak bloom and natural populations of pollinators; tunnels also impose a physical barrier to pollination insects. If plastic tunnels are selected as the preferred management system, a well recommended practice is to provide bumblebee hives inside the hoops in order to assure a good ratio of pollinators

Table 4. Matrix classification of blueberry fruits according to cultivar [(a,b)]

True category	Predicted category		
	'O'Neal'	'Sharpblue'	'Misty'
'O'Neal'	85.7%	14.3%	0.0%
'Sharpblue'	6.7%	93.3%	0.0%
'Misty'	0.0%	50.0%	50.0%

[a] 81.1% of original grouped cases correctly classified. [b] 78.4% of cross-validated grouped cases correctly classified.

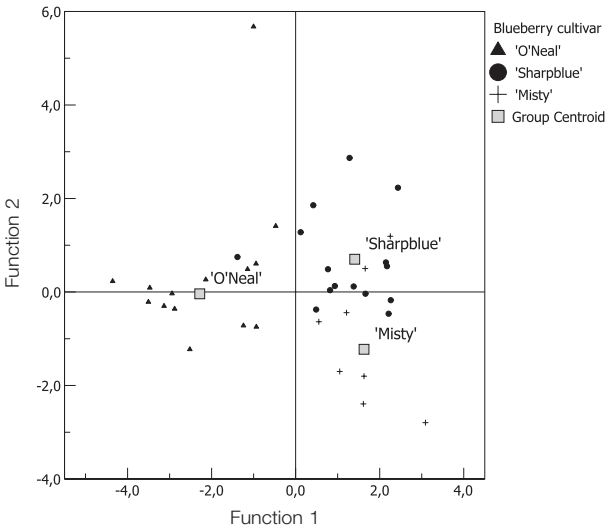

Figure 2. Scatter plot of the canonical scores from the stepwise discriminant analysis.

(Mackenzie, 1997; Williamson and Lyrene, 2004). Firmness is one of the most important parameter of quality for blueberries produced for fresh market. Berry firmness affects fruit shelf life, and it is related to marketing, consumer acceptance and to post harvest decay of fruit (Donahue *et al*., 1999; Parra *et al*., 2007). Microclimatic conditions during harvest in protected plots seems not to affect this parameter. Ehlenfeldt and Martin (2002) reported higher mean values for 'Misty' and 'O'Neal', as also did Zee *et al.* (2006) for 'Misty'; differences that may be expected due to the methodology employed and local growing conditions.

Berries to be consumed fresh should be selected for high balanced soluble solids and acid content. Processing blueberries require higher acidity than those for fresh consumption because the typical tart blueberry flavour is diluted on mixing. In both aspects, results suggest that 'Sharpblue' and 'Misty' are superior to 'O'Neal', which had a low sugar, and low titratable acidity which may be too bland for most palates; also, such fruits do not keep well. Results reflect that the main effect on fruit quality parameters measured was cultivar; production system did not explain much of the differences observed in the physico-chemical characteristics of fruits. Remberg *et al.* (2006) reported quality differences among cultivars from American and European origin and Ballington *et al.* (1984) concluded that, within a single location and year, genetic components are undoubtedly more important than environmental differences within the field. Variations in chemical composi-

tion may occur as a result of judging the maturity of berries by blue coloration (Connor *et al*., 2002). Organoleptic characteristics can also vary between areas, thus we think that it is interesting to have this kind of data to provide a comparative basis.

We do not intend here to conclude what blueberry cultivar is better or what production system is most effective. Both choices will result from a market survey of the final destination of production (viz. exportation, industry, fresh pack market, etc..), and from the analysis of many other factors, related with the economics of growers, socio-economic variables that need not coincide with the ones reported.

To summarise, blueberry culture in Andalusia is characterised by interesting and growing market opportunities. In this study, cultivar genetics seems to be the main variation source for the physical and chemical variables measured increasing the importance of a good cultivar selection well adapted to each location. Main differences were restricted to bigger fruits for plants growing in open air, and no significant differences in parameters measured were found when blueberry cultivars were assessed under two management systems. Future studies should include additional cultivars and locations to obtain a complete profile of the fruit quality of Andalusian blueberries.

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