Some factors that may affect the physical-chemical properties of blueberries

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Abstract: The aim of the present study was to investigate the effect of different production and conservation factors on some properties of blueberries. Among the production factors considered were cultivar (Duke, Bluecrop and Ozarkblue) and production mode (organic or conventional). Regarding the conservation factors were evaluated temperature (ambient or refrigeration) and storage time (0, 7 and 14 days). The properties under study belong to three categories: physical properties (color and texture); chemical properties (moisture content, sugars and acidity) and phenolic and antioxidant properties (total phenols, anthocyanins, tannins, ABTS antioxidant activity, DPPH antioxidant activity).

The results revealed that moisture content was only influenced by cultivar and that both acidity and sugar contents varied according to the production mode used. Also it was evidenced that the antioxidant activity was not statistically different between cultivars, production modes or conservation conditions. Regarding the phenolic compounds, the tannins were significantly higher for the blueberries produced in organic agriculture. Regarding color significant differences were also encountered and the most intense blue was found in blueberries from cv. Duke, produced in organic farming and stored under refrigeration. Textural attributes were also very significantly influenced by all factors at study: cultivar, production mode and conservation, and the berries from cv. Duke stored under refrigeration showed the highest firmness.

Keywords: blueberry, cultivar, conservation, production mode, statistical analysis

Citation: Guiné, R. P. F., C. F. Gonçalves, F. Gonçalves, and D.V. T. A. Costa. 2016. Some factors that may affect the physical-chemical properties of blueberries. Agricultural Engineering International: CIGR Journal, 18(3):334-342.

1 Introduction

Blueberries (*Vaccinium* spp.) are very popular fruits all over the world, being extremely appreciated due to their nutritive components, including vitamins, anthocyanins, mineral elements, and flavonols (Xu et al. 2016). The quality of blueberries is to a great extent related to the levels of phenolic compounds and, consequently, to their antioxidant activity, being among the foods with the highest antioxidant properties (Gündüz, Serçe, and Hancock 2015; Rodrigues et al. 2011). Their consumption has increased in the past decades due to a higher consumer demand originated greatly by their perceived health benefits (Retamales et al. 2015).

Traditional or conventional agriculture uses large amounts of fertilizers and chemical plant protection products to intensify the production and increase the production yields, making it possible to produce enough food to meet global needs at relatively low costs. However, these practices have proven harmful to the environment and lead, eventually, to the degradation of ecosystems, thus creating a serious threat to the quality of life of all living beings. Contrarily, the organic farming seeks to integrate a set of agricultural techniques aiming at a rational use of the ecosystems (comprising the climate, water, soil, microorganisms, plants, animals) so as to preserve the balance and sustainability at long term. Hence, organic farming corresponds to an

Received date: 2016-05-24 **Accepted date:** 2016-07-04

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environmental-friendly set of practices that can counter the negative effects of agricultural intensification and the decline of biodiversity in agricultural landscapes. This agricultural production system excludes almost all synthetic chemicals, such as fertilizers or pesticides. Organic farming promotes a sustainable food production through application of multiple types of organic fertilizers and reduction of pesticide utilization. Long-term effects of organic farming generally result in higher organic matter levels, increased soil biodiversity and aboveground pest suppression (Sandhu, Wratten, and Cullen 2010; Chamorro, Masalles, and Sans 2016; Quist et al. 2016).

During storage a substantial number of physical and chemical changes happen in the agricultural products in general and blueberries in particular. These alterations can meaningfully influence the quality of the berries, originating senescence and death. The transportation and storage times are relatively limited principally because of the fungus and the tendency of the blueberries to dehydrate. To a large extent, the quality and storage life of blueberries is related to the soluble solids content, acidity and ratio of soluble solids to acidity. Usually, blueberries are stored in freezers at temperatures between 2 ℃ and 4 ℃ and 85% to 90% relative humidity, for a period of up to 10 days, depending on the cultivar (Retamales et al. 2015; Severo et al. 2009; Galarça et al. 2012).

The aim of the present study was to evaluate the effect of different production and conservation factors on the properties of blueberries. The production factors considered were cultivar (Duke, Bluecrop and Ozarkblue) and production mode (organic or conventional). The conservation factors studied were temperature (ambient or refrigeration) and time (0, 7 and 14 days). The properties under study fall into three categories: physical properties (color and texture); some chemical components (moisture content, sugars and acidity) and phenolic and antioxidant properties (total phenols, anthocyanins, tannins, ABTS antioxidant activity, DPPH antioxidant activity).

2 Experimental procedure

2.1 Harvesting and conservation

In the resent study blueberries form three cultivars were analyzed (Duke, Bluecrop, Ozarkblue), original from conventional and organic production modes. The fruits were harvested at a maturity state corresponding to the marketing conditions, according to the producers. The properties were evaluated in the fresh samples (corresponding to 0 day of storage) and also after 7 and 14 d of storage under refrigeration at a temperature of 4 ℃ and 85% to 90% relative humidity (RH).In addition, for the cultivar Duke produced in conventional mode, was also tested the storage at room temperature (around 15 ℃ to 25 ℃ and 30% to 60% RH).

2.2 Chemical analysis

For the evaluation of moisture content a Halogen Moisture Analyzer HG53 from Mettler Toledo was used. Acidity was determined by titration according to the Portuguese Standard NP-1421 and total soluble solids, corresponding approximately to the sugar content expressed in sucrose, were determined in °Brix using a refractometer Atago 3T.

To extract the phenolic compounds from the samples at study, methanol and acetone solutions were used, so that in each case the sample was left for 1 h in an ultrasonic bath at room temperature to contact with the extraction solution. The extracts obtained were then used to quantify the phenolic composition and the antioxidant activity. The total phenolic compounds were determined by the Folin–Ciocalteu method. Total anthocyanins were determined using the SO_2 bleaching method. Total tannins were estimated by modification of method described by Ribereau-Gayon and Stonestreet. The antioxidant activity was determined by DPPH and ABTS methods(Guiné, Soutinho, and Gonçalves 2014).

2.3 Analysis of the physical properties

The color coordinated were determined using a colorimeter (Chroma Meter - CR-400, Konica Minolta) in the CIE Lab color space, measuring the Cartesian coordinates L*, a* and b*,where L* is brightness (0 =

black to 100 = white), a* is green/red (negative or positive values, respectively), and b* is blue/yellow (negative or positive values, respectively).

To determine the texture attributes (firmness and elasticity) the evaluations were performed with a texturometer TA.XT Plus (Stable Micro Systems).The test measured the compression force using a probe of 2 mm (P/2), with the following test conditions: pre-test speed = 1.50 mm/s, test-speed = 1.00 mm/s, post-test speed = 1.00 mm/s, distance = 6 mm, trigger force = 0.05 mm and a load cell of 50 kg. The results were processed using Exponent software TEE (Stable Micro Systems) and from the obtained texture profiles were determined the firmness (the strength at the highest peak) and elasticity (distance at the highest point).

2.4 Statistical analysis

For the treatment of the data were performed different tests, according to the case applicable for each set of variables. For the comparison of all variables according to the cultivar the Kruscal Walis test was used. For the comparison of the properties in relation to the production mode in some cases the t test for independent samples was used and in others was used the U Mann Whitney test. For the conservation conditions the U Mann Whitney test was always used.

Also the Pearson coefficients (r) were determined between the quantitative variables to evaluate the intensity of the relations between the properties evaluated. If $0.00 < r < 0.10$ the association is considered very weak, if $0.10 \le r < 0.30$ the association is weak, if $0.30 \le r < 0.50$ the association is moderate, if $0.50 \le r < 0.70$ the association is strong and if $0.70 \le r < 1.00$ the association is very strong. For $r = 0$ there in no association and for $r = 1$ the association is perfect.

In all tests a level of significance of 95% was considered and the software used for all tests was SPSS version 21 (IBM Inc.).

3 Results and discussion

3.1 Effect of cultivar on the physical-chemical properties

Table 1 shows the results obtained through the Kruskal-Wallis tests made to the effect of cultivar on the chemical composition. The results show that there are significant differences ($p = 0.045$), at the significance level of 5%, in moisture content, and that the moisture is higher for the cultivar Duke, then decreasing in the order Ozarkblue and Bluecrop. Regarding acidity and sugars, no significant differences were observed among cultivars ($p = 0.089$ and $p = 0.083$, respectively).The differences in moisture content observed for some cultivars may be attributed to the cultivar itself, or may also to some extent be related either to different maturity stages of the fruits, since the fruits were harvested by the producer without an objective evaluation of the maturity stage. Other factor that may contribute for the differences in moisture could be the quantity of rain or the irrigation intensity of each of the plantations.

Table1 Kruskal-Wallis test between cultivar and the chemical components

Property	Duke		Bluecrop		Ozarkblue		χ^2	p-value
	N	Mean Rank	N	Mean Rank	N	Mean Rank		
Moisture, g water/100 g	36	38.69	8	19.75	24	33.13	6.188	0.045
Acidity, mg citric acid/100 g	27	32.87	6	31.58	18	22.17	4.832	0.089
Sugars, g sucrose/100 g	27	22.70	6	22.00	18	32.28	4.973	0.083

The results of the Kruskal-Wallis tests in Table 2 show that none of the phenolic properties considered (total phenols, tannins, anthocyanins or antioxidant activity by both methods) varied among the different cultivars studied, since in all cases the p-value was higher than 0.05, hence assuming the null hypothesis of absence of significant differences. This results was surprising, because studies report that there is considerable variation in the phenolic profiles and antioxidant capacities among cultivars and species of blueberries (Cardeñosa et al. 2016). The fact that these cultivars showed similar phenolic compounds contents and antioxidant properties may be due to some production factors, such as temperature, sun incidence or hydric stress, which greatly contribute for the development of phenolic compounds in plant materials as a response to environmental stress conditions(Cardeñosa et al. 2016).

Table 2 Kruskal-Wallis test between cultivar and the phenolic properties

Property	Duke		Bluecrop		Ozarkblue		χ^2	p-value
	N	Mean Rank	N	Mean Rank	N	Mean Rank		
DPPH Antioxidant activity, µmol trolox/g	108	104.76	24	95.98	72	101.28	0.482	0.786
ABTS Antioxidant activity, µmol trolox/g	108	105.83	24	113.75	72	93.76	2.795	0.247
Total phenols, µg galic acid eq./g	108	100.40	24	102.46	72	105.66	0.343	0.843
Tannins, mg/g	108	99.62	24	118.29	72	101.56	1.994	0.369
Anthocyanins, mg malvidin-3-glucoside/g	108	107.79	24	102.50	72	94.57	2.174	0.337

Table 3 presents the results of the Kruskal-Wallis test between cultivar and the color and textural properties. The results indicate that in all cases, for a level of significance of 5%, there are highly significant differences, as indicated by the p-velues. The results show that lightness was higher for cultivar Ozarkblue, indicating that these berries were clearer, then Bluecrop and finally cv. Duke, which had the lowest value of L*, and hence was the darkest. As to the coordinate a*, its value was higher for cv. Bluecrop, then for Ozarkblue and lastly for Duke. On the contrary, the values of b* varied in the inverse relation, so that b* was higher for cv. Duke, then Ozarkblue and finally Bluecrop. Because the values of b* are negative, indicative of the presence of blue instead of yellow, increasing b* means that the blue is less intense. Hence, in this case, the least intense blue was found on cv. Duke. The blue coloration is derived from the contents in some natural pigments, namely anthocyanins, and therefore may vary according to its formation intensity along the maturation of the fruits as well as post-harvest conditions (Martynenko and Chen 2016).

Table 3 Kruskal-Wallis test between cultivar and the physical properties

Property	Duke		Bluecrop		Ozarkblue		χ^2	p-value
	N	Mean Rank	N	Mean Rank	N	Mean Rank		
L*, lightness	495	357.74	110	496.11	330	624.02	193.853	0.000
a*, green/red	495	396.08	110	575.57	330	540.02	76.034	0.000
b*, blue/yellow	495	575.78	110	338.47	330	349.51	167.673	0.000
Firmness, N	470	533.59	107	304.70	323	377.89	106.885	0.000
Elasticity, mm	470	576.12	107	442.27	323	270.44	264.833	0.000

In relation to texture, it was observed that both firmness and elasticity were higher for cv. Duke and lower for cv. Ozarkblue (Table 3). The textural characteristics of the fruits are somewhat correlated to their moisture content as well as fiber content, and therefore it is expected that differences in moisture could also be associated with differences in texture.

3.2 Effect of production mode on the physical-chemical properties

Table 4 shows the results of the U Mann-Whitney tests made between the production mode and the chemical parameters analyzed. The results indicated that moisture content was not significantly different among the two production modes studied (conventional or organic) ($p = 0.077$) at the level of significance of 5%. However, both acidity and sugars content varied significantly according to the production mode ($p < 0.001$ in both cases), so that organic berries had higher acidity and lower sugar contents.

Table 4 U Mann-Whitney test between production mode and the chemical components

Property	Conventional		Organic		UMW	p-value
	N	Mean Rank	N	Mean Rank		
Moisture, g water/100 g	40	37.36	8	30.41	445.50	0.077
Acidity, mg citric acid/100 g	30	13.20	21	25.71	99.00	0.000
Sugars, g sucrose/100 g	30	33.25	21	15.64	97.50	0.000

The effect of production mode on the phenolic properties was studied by means of the independent samples t-test, whose results are presented in Table 5. The results showed that there are no significant differences between the antioxidant activity in the berries from conventional or organic production modes, regardless of the method used to quantify antioxidant activity ($p > 0.05$ in both cases). Also total phenols content was not significantly different, at the level of significance of 5%, among production modes ($p = 0.264$), and the same was observed for anthocyanins ($p = 0.455$). Conversely, the tannins content was significantly different ($p = 0.002$), being higher in the berries from organic farming. The stress conditions provoked by the organic production mode lead the plant material to produce some phenolic compounds in higher amounts, and this could be the case with the tannins.

Table 5 Independent samples t-test between production mode and the phenolic properties

Property	Conventional			Organic			Levene	t	p-value
	N	Mean	St.Dev.	N	Mean	St.Dev.			
DPPH Antioxidant activity, µmol trolox/g	120	2.281	0.260	84	2.550	0.344	0.293	-0.635	0.263
ABTS Antioxidant Activity, µmol trolox/g	120	1.990	0.268	84	2.332	0.362	0.096	-0.775	0.220
Total phenols, µg galic acid eq./g	120	1.617	0.155	84	1.771	0.189	0.990	-0.634	0.264
Tannins, mg/g	120	0.499	0.037	84	0.765	0.080	0.000	-3.006	0.002
Anthocyanins, mg malvidin-3-glucoside/g	120	0.500	0.071	84	0.512	0.085	0.402	-0.113	0.455

Table 6 shows the results of the independent samples t-test between production mode and the physical properties, namely color coordinates and textural parameters. It was observed that lightness (L*) varied highly significantly with production mode ($p < 0.001$), so that the berries from organic farming were lighter (with higher L*). Greenness/redness (a*) showed no significant differences for both production modes studied ($p = 0.315$), but blueness (b*) showed highly significant differences ($p < 0.001$), being lower for organic berries, which therefore presented a more intense blue, at the level of significance of 5%.The color of the blueberries is associated with the presence of pigments such as anthocyanins, and therefore the differences between productions modes could be expected.

Table 6 Independent samples t-test between production mode and the physical properties

Property	Conventional			Organic			Levene	t	p-value
	N	Mean	St.Dev.	N	Mean	St.Dev.			
L*, lightness	550	33.334	3.568	385	36.180	3.246	0.000	-12.659	0.000
a*, green/red	550	0.371	0.972	385	0.414	1.537	0.000	-0.482	0.315
b*, blue/yellow	550	-5.877	1.638	385	-7.245	2.222	0.002	10.131	0.000
Firmness, N	526	1.587	0.357	374	1.632	0.371	0.306	-1.796	0.037
Elasticity, mm	526	2.862	0.711	374	2.689	0.816	0.001	3.314	0.001

Firmness showed significant differences among production modes (p = 0.037), being the berries from organic farming harder than those from conventional production mode. In regards to elasticity, very significant differences were observed (p = 0.001) and the fruits from conventional agriculture were the more elastic. The higher resistance of the organic blueberries constitutes an advantage for handling and conservation and therefore proves some benefits of using organic farming instead of conventional agriculture. Some identified key challenges that could also contribute for the expansion of certified organic blueberry production include greater production costs and/or inputs (particularly for fertilization and weed management), limited options for disease and insect control associated with greater risk, and reduced yields of organic plantings (whether perceived or real) (Strik 2016).

3.3 Effect of conservation on the physical-chemical properties

The postharvest shelf-life of fresh blueberries is quite short when they are kept at room temperature because they undergo mechanical damage, microbial growth, and nutritional and moisture loss which seriously reduces commercial value of blueberries (Xu et al. 2016). Hence the effect of conservation conditions on the properties of the berries is crucial. Table 7 shows the results of the U Mann-Whitney tests between conservation conditions and the chemical components evaluated. The results indicated that in all cases, at the level of significance of 5%, neither of the properties studied (moisture, acidity or sugar content) varied significantly according to the conditions used for storage (ambient temperature or refrigeration). These results are very interesting given that blueberries are perishable and from the moment that they are harvested they are very susceptible to structural, nutritional and biochemical changes (Vieira et al. 2016).

Table 7 U Mann-Whitney test between conservation temperature and the chemical components

Property	Ambient temp.		Refrigeration		UMW	p-value
	N	Mean Rank	N	Mean Rank		
Moisture, g water/100 g	12	41.08	56	33.09	257.00	0.102
Acidity, mg citric acid/100 g	9	25.50	42	26.25	157.50	0.216
Sugars, g sucrose/100 g	9	29.39	42	25.27	158.50	0.226

Table 8 shows the results of the U Mann-Whitney tests between conservation conditions and the phenolic composition or antioxidant activity. It was observed that, at the level of significance of 5%, only the tannins content showed significant differences according to the conservation conditions (p = 0.031), being higher when the berries were stored under refrigeration.

Table 8 U Mann-Whitney test between conservation temperature and the phenolic properties

Property	Ambient temp.		Refrigeration		UMW	p-value
	N	Mean Rank	N	Mean Rank		
DPPH Antioxidant activity, μmol trolox/g	36	99.36	168	103.17	2911.00	0.363
ABTS Antioxidant activity, μmol trolox/g	36	94.92	168	104.13	2751.00	0.198
Total phenols, μg galic acid eq./g	36	89.81	168	105.22	2567.00	0.078
Tannins, mg/g	36	85.76	168	106.09	2421.50	0.031
Anthocyanins, mg malvidin-3-glucoside/g	36	103.82	168	102.22	2976.50	0.441

The results in Table 9 indicate that except for a*, whose p-value was higher than 0.05, all other physical properties revealed highly significant differences according to the conservation conditions ($p < 0.001$). In this way it was confirmed that the berries stored under refrigeration had higher L* and lower b*, and hence were clearer and with more intense blue. Also the refrigerated blueberries had higher firmness and were less elastic. The alteration of the physical characteristics of blueberries are strictly related to their susceptibility to spring frosts and degree of winter freezing tolerance, which have been identified as two of the most important genetic limitations of current blueberry cultivars (Die, Arora, and Rowland 2016).

Table 9 U Mann-Whitney test between conservation temperature and the physical properties

Property	Ambient temp.		Refrigeration		UMW	p-value
	N	Mean Rank	N	Mean Rank		
L*, lightness	165	277.61	770	580.80	32110.00	0.000
a* green/red	165	441.34	770	473.71	59126.50	0.081
b*,blue/yellow	165	687.11	770	421.05	27371.50	0.000
Firmness, N	158	366.43	742	438.40	45335.00	0.000
Elasticity, mm	158	516.66	742	436.41	48165.00	0.000

Table 10 shows the Pearson correlation coefficients between storage time and the physical-chemical properties studied. The results indicate that no meaningful associations were found between storage time and these properties, with the highest value being 0.381, which reflects a moderate positive association between storage time and elasticity, so that as time increases elastic also increases.

Table 10 Pearson correlations between storage time and the physical-chemical properties

Property	Pearson correlation coefficient,r
Moisture	0.057
Acidity	0.194
Sugars	0.188
DPPH Antioxidant activity	-0.030
ABTS Antioxidant activity	0.007
Total phenols	0.058
Tannins	-0.300
Anthocyanins	0.028
L* ,lightness	-0.183**
a*,green/red	0.082*
b*,blue/yellow	0.196**
Firmness	0.162**
Elasticity	0.381**

Note: **Correlation is significant at the 0.01 level.

*Correlation is significant at the 0.05 level

3.4 Correlations between the physical-chemical properties

Table 11 shows the Pearson correlation coefficients between the physical properties, color and texture. Although in most cases the correlations found are significant at the level of 5%, they are very weak, with just the exception of the correlation between b* and L*, which is strongly negative (r = -0.706), indicating that the blueness and the darkness are strongly inversely correlated.

Table 11 Pearson correlations between the physical properties

	DPPH	ABTS	TP	TAN	ANT
DPPH	1				
ABTS	0.958**	1			
TP	0.871**	0.920**	1		
TAN	0.579**	0.586**	0.616**	1	
ANT	0.865**	0.917**	0.947**	0.500**	1

Note: L*: Lightness, a*: Green/red, b*: blue/yellow, FR: Firmness, EL: Elasticity.

**Correlation is significant at the 0.01 level.

The data in Table 12 shows that in all cases the Pearson correlation coefficients between the phenolic composition and the antioxidant activity were significant al the level of significance of 5%. Furthermore, it was observed that ABTS and DPPH antioxidant activities are very strongly positively correlated (r = 0.958). Also the antioxidant activity is very strongly correlated with total phenols: r = 0.871 for DPPH method and r = 0.920 for ABTS method. This shows that the presence of phenolic compounds strongly contributes for the antioxidant activity of the blueberries. The tannins content are moderately positively associated with antioxidant activity (r = 0.579 and r = 0.586, respectively for DPPH and ATS methods), also moderately correlated with total phenols (r = 0.616) and moderately correlated with anthocyanins (r = 0.500). On the other hand, anthocyanins are very strongly correlated with antioxidant activity (r = 0.865 and r = 0.917, respectively for DPPH and ATS methods) and with total phenols (r = 0.947), indicating that anthocyanins are important constituents of the blueberries analyzed and that they contribute decisively for their antioxidant activity.

Table 12 Pearson correlations between the phenolic properties

	L*	a*	b*	FR	EL
L*	1				
a*	-0.210**	1			
b*	-0.706**	0.241**	1		
FR	-0.134**	0.040	0.120**	1	
EL	-0.274**	-0.089**	0.145**	0.327**	1

Note: DPPH: DPPH Antioxidant activity, ABTS: ABTS Antioxidant Activity, TP: Total phenols, TAN: Tannins, ANT: Anthocyanins.

**Correlation is significant at the 0.01 level.

4 Conclusions

This work allowed concluding that moisture content in blueberries was only influenced by cultivar, whereas acidity and sugar contents were influenced by production mode.

Regarding the antioxidant activity no statistically significant differences were found according to cultivar, or production mode, or even conservation conditions. As to the phenolics, only tannins showed significant differences according to production mode, being higher for organic farming.

Color parameters lightness and blueness were found to vary significantly according to cultivar, production mode and conservation conditions. The most intense blue was found in blueberries from cv. Duke, for production in biological mode and for storage under refrigeration.

Texture was also very significantly influenced by the three factors at study, cultivar, production mode and conservation, being the berries with higher firmness those from cultivar Duke and stored under refrigeration.

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