

Factors affecting blueberry physical-chemical properties

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Abstract

The objective of this work is to investigate the effect of different production and conservation factors on the properties of blueberries. The production factors considered were cultivar (Duke, Bluecrop, Ozarkblue) and production mode (organic or conventional). The conservation factors were temperature (ambient, refrigeration) and time (0, 7, 14 days). The properties under study were: 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). This work showed that moisture content was only influenced by cultivar and that both acidity and sugar contents varied according to the production mode used. The results evidenced that the antioxidant activity was not statistically different according to cultivar, production mode or conservation. As to the phenolics, only tannins showed significant differences according to production mode, being higher for organic farming. Color parameters showed some significant differences, so that the most intense blue corresponded to blueberries from cv. Duke, from organic farming and stored 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.

Keywords

Blueberry, Cultivar, Conservation, Production Mode, Statistical Analysis.

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Conference Topic - TS1 - Food Engineering and Properties

1. Introduction

Blueberries are largely consumed perishable fruits with recognized healthy nutrients [1]. Their consumption has increased in the past decades, most of it owing to the interest in their production given the consumer demand due to their perceived health benefits [2]. Some limits for specific physical-chemical components of blueberries were proposed by Beaudry [3]: acidity varying from 0.3 to 1.3% (w/w), soluble solids over 10% (w/w) and sugar: acid ratios between 10 and 33. However, many authors have found different values [4-6].

The quality of blueberries has become associated with the levels of phenolic compounds as well as their antioxidant activity, being among the foods with the highest antioxidant properties [6,7].

Conventional agriculture, using large amounts of fertilizers and plant protection products made it possible to produce enough food to meet global needs. However, these practices led to environmental damage and degradation of ecosystems, which poses a serious threat to the quality of life of all living beings. In contrast to conventional agriculture there is organic farming, which integrates a set of agricultural techniques for the rational use of the system formed by the climate - water - soil microorganisms - plant, in order to preserve the balance of agricultural ecosystems and make them sustainable in the long term. In this agricultural production mode are excluded almost all the synthetic chemicals, such as chemical fertilizers and synthesis pesticides. This mode of production is more environmentally friendly and sustainable, by harmonizing the practices with natural balances, the increased use of renewable inputs, low energy cost, and for preventing practices and greater environmental impact products [8].

A considerable number of physico-chemical changes occur during the storage of fruits in general and blueberries in particular, which can significantly reduce the quality of the berries, leading to senescence and death over the same time. The storage time and the time required to transport the blueberries are limited mainly because of the fungus and the easy ability of the blueberry to dehydrate. To a large extent, the quality and storage life of fruit blueberries is related to the soluble solids content, acidity and ratio of soluble solids to acidity. The blueberries are stored in freezers at temperatures between 2 and 4 °C and 85 to 90% relative humidity. Usually the fruits are kept for a period of 10 days, depending on the cultivar [2,9,10].

The objective of this work is to assess 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

Blueberries from three cultivars were analyzed in this study (Duke, Bluecrop, Ozarkblue), produced in conventional and organic modes. The fruits were harvested at market maturity state. The properties were evaluated in the fresh samples and also after 7 and 14 days of storage under refrigeration at a temperature of 4 °C 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 °C and 30 to 60% RH).

2.2. Chemical analysis

Moisture content was determined by a Halogen Moisture Analyzer HG53 from Mettler Toledo. Acidity was determined by titration according to the Portuguese Standard NP-1421. Total sugars were determined by refractometry using a refractometer Atago 3T.

The phenolic compounds were extracted with methanol and with acetone solutions, and in each case the sample was left for 1 hour in an ultrasonic bath at room temperature. The extracts obtained were then used to quantify the phenolic composition and the antioxidant activity. The total phenolic content in the fruit extracts was determined by the Folin-Ciocalteu method. Total anthocyanins were determined using the SO₂ 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.

2.3. Analysis of the physical properties

The color was determined with a colorimeter (Chroma Meter - CR-400, Konica Minolta) in the CIE Lab color space, through 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) and the results were treated with software Exponent TEE.

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 Kruskal-Wallis 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 $0.10 \leq r < 0.30$ the association is weak, if $0.30 \leq r < 0.50$ the association is moderate, if $0.50 \leq r < 0.70$ the association is strong and if $0.70 \leq r < 1.00$ the association is very strong. For $r = 0$ there is 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).

Table 1 - Kruskal-Wallis test between cultivar and the chemical components.

Property	Duke		Bluecrop		Ozarkblue		χ^2	Sig. (p-value)
	N	Mean Rank	N	Mean Rank	N	Mean Rank		
Moisture	36	38.69	8	19.75	24	33.13	6.188	0.045
Acidity	27	32.87	6	31.58	18	22.17	4.832	0.089
Sugars	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.

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

Property	Duke		Bluecrop		Ozarkblue		χ^2	Sig. (p-value)
	N	Mean Rank	N	Mean Rank	N	Mean Rank		
DPPH Antioxidant activity	108	104.76	24	95.98	72	101.28	0.482	0.786
ABTS Antioxidant Activity	108	105.83	24	113.75	72	93.76	2.795	0.247
Total phenols	108	100.40	24	102.46	72	105.66	0.343	0.843
Tannins	108	99.62	24	118.29	72	101.56	1.994	0.369
Anthocyanins	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-values. 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. In relation to texture, it was observed that both firmness and elasticity were higher for cv. Duke and lower for cv. Ozarkblue (Table 3).

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

Property	Duke		Bluecrop		Ozarkblue		χ^2	Sig. (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	470	533.59	107	304.70	323	377.89	106.885	0.000
Elasticity	470	576.12	107	442.27	323	270.44	264.833	0.000

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	Sig. (p-value)
	N	Mean Rank	N	Mean Rank		
Moisture	40	37.36	8	30.41	445.50	0.077
Acidity	30	13.20	21	25.71	99.00	0.000
Sugars	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.

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

Property	Conventional			Organic			Levene	t	Sig. (p-value)
	N	Mean	St.Dev.	N	Mean	St.Dev.			
DPPH Antioxidant activity	120	2.281	0.260	84	2.550	0.344	0.293	-0.635	0.263
ABTS Antioxidant Activity	120	1.990	0.268	84	2.332	0.362	0.096	-0.775	0.220
Total phenols	120	1.617	0.155	84	1.771	0.189	0.990	-0.634	0.264
Tannins	120	0.499	0.037	84	0.765	0.080	0.000	-3.006	0.002
Anthocyanins	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%.

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.

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

Property	Conventional			Organic			Levene	t	Sig. (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	526	1.587	0.357	374	1.632	0.371	0.306	-1.796	0.037
Elasticity	526	2.862	0.711	374	2.689	0.816	0.001	3.314	0.001

3.3. Effect of conservation on the physical-chemical properties

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).

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

Property	Ambient temp.		Refrigeration		UMW	Sig. (p-value)
	N	Mean Rank	N	Mean Rank		
Moisture	12	41.08	56	33.09	257.00	0.102
Acidity	9	25.50	42	26.25	157.50	0.216
Sugars	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	Sig. (p-value)
	N	Mean Rank	N	Mean Rank		
DPPH Antioxidant activity	36	99.36	168	103.17	2911.00	0.363
ABTS Antioxidant Activity	36	94.92	168	104.13	2751.00	0.198
Total phenols	36	89.81	168	105.22	2567.00	0.078
Tannins	36	85.76	168	106.09	2421.50	0.031
Anthocyanins	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.

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

Property	Ambient temp.		Refrigeration		UMW	Sig. (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	158	366.43	742	438.40	45335.00	0.000
Elasticity	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**

**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.

	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

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 at 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.

	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

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

**Correlation is significant at the 0.01 level. *Correlation is significant at the 0.05 level.

4. Conclusions

This work allowed concluding that moisture content in blueberries was only influenced by cultivar, whereas acidity and sugars 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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