

Statistical Analysis of the Factors that Influence the Properties of Blueberries from Cultivar Bluecrop

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Abstract—Because blueberries are worldwide recognized as a good source of beneficial components, their consumption has increased in the past decades, and so have the scientific works about their properties. Hence, this work was undertaken to evaluate the effect of some production and conservation factors on the properties of blueberries from cultivar Bluecrop. The physical and chemical analyses were done according to established methodologies and then all data was treated using software SPSS for assessment of the possible differences among the factors investigated and/or the correlations between the variables at study. The results showed that location of production influenced some of the berries properties (caliber, sugars, antioxidant activity, color and texture) and that the age of the bushes was correlated with moisture, sugars and acidity, as well as lightness. On the other hand, altitude of the farm only was correlated to sugar content. With regards to conservation, it influenced only anthocyanins content and DPPH antioxidant activity. Finally, the type of extract and the order of extraction had a pronounced influence on all the phenolic properties evaluated.

Keywords—Antioxidant activity, blueberry, conservation, geographical origin, phenolic compounds, statistical analysis.

I. INTRODUCTION

IN Portugal, the blueberry crop has assumed leadership and gained interest from both consumers and producers. The growth and development of the food sector, and the influence of other countries in Europe, introduced new eating habits, such as consumption of functional foods, and particularly red fruits.

The consumption of berries has become popular among consumers concerned about health because of their high levels of bioactive compounds with antioxidant properties [1], [2]. Within existing antioxidants in blueberry, those which play a more important role are the phenolic compounds, including flavonoids, anthocyanins and tannins, making it one of the most desirable and nutritious fruits [3]. As a high source of bioactive compounds, blueberry emerged as with high therapeutic potential for slowing the progression of various diseases [4]. This small berry, in addition to its protective

effects in the intestinal tract and eyes, acts in the principal anti-inflammatory mechanisms and oxidative stress. Furthermore there have been described several beneficial properties associated with blueberries, in particular for treating and preventing diseases of the nervous system [5], urinary tract disorders, cardiovascular diseases, dental caries, ulceration and gastric cancer [3]. Also described are anti-proliferative, anti-cancer, antiviral and antibacterial activities [6], [7].

Despite the high content in phytochemicals, in most varieties of blueberries, it is known that either the amount or the exact profile of these compounds may vary, not only with the cultivar and the degree of maturation [8], but also with the conditions of soil and climate or agricultural practices, as well as conservation [9]. Some of these differences may be the result of different degrees of water stress, the greater or lesser availability of nutrients and intensity of ultraviolet light [10], [11]. A considerable variability in the phenolic profile and antioxidant capacity of different *Vaccinium* species has been reported in literature [12]–[14].

Portugal, due to the variety of its reliefs and exposure of its slopes and valleys, presents very different microclimate conditions, which may influence the blueberries properties [15].

The aim of this work was to evaluate the influence of production and conservation factors as well as extraction conditions on the physical-chemical properties of blueberries from cultivar Bluecrop. The production factors considered were origin (5 different locations were considered), altitude of the farm placement and age of the bushes. The conservation factor studied was storage under freezing as opposed to the fresh product. The extraction factors considered were type of solvent used and order of the extraction. The physical properties under study were caliber, color and texture; the general chemical properties were moisture content, sugars and acidity; the phenolic and antioxidant properties included total phenols, anthocyanins, tannins and antioxidant activity (measured by three methods: ABTS, FRAP, DPPH).

II. MATERIALS AND METHODS

A. Samples

To carry out this study were used blueberries *Vaccinium corymbosum* L., from cultivar Bluecrop grown in conventional production mode in five different locations of Portugal: Sever do Vouga (SV), Estarreja (ES), Oliveira do Hospital (OH), Vouzela (VZ) and Braga (BR). The fruits were manually collected in a maturation state corresponding to commercialization.

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The analyses were made right after harvest in the fresh fruits and also after 6 months of storage under freezing at a temperature of -20°C.

B. Chemical Analyses

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 according to [16]. Total anthocyanins were determined using the SO₂ bleaching method [17]. Total tannins were estimated by modification of method described by [18]. The antioxidant activity was determined by DPPH [19], ABTS [20] and FRAP [21], [22] methods.

C. Physical Properties Evaluation

The size of each berry was measured with the aid of an automated caliper rule.

The color was determined with a colorimeter (Chroma Meter - CR-400, Konica Minolta) in the CIE Lab color space, though 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.

D. Statistical Analysis

For the treatment of the data were performed different tests, according to the case applicable for each set of variables, and the verification or not of the conditions to apply parametric tests. For the comparison according to the origin of production Kruskal-Wallis test was used for the caliber and chemical analyses and One-way Anova was used for color and texture, with multiple comparisons by the Tukey test.

For the comparison of the properties in relation to the conservation conditions the t test for independent samples was used.

For the extraction solvent the t test for independent samples was always used and for the order of the extraction One-way ANOVA with Tukey multiple comparisons was applied. Also the Pearson coefficients (r) were determined between the quantitative. The Pearson correlation coefficient measures the association between two variables according to the magnitude of the absolute value [23]–[25]. 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.).

III. RESULTS AND DISCUSSION

A. Effect of Origin on the Blueberry Properties

Table I shows the results of the Kruskal-Wallis test made to verify the effect of origin of production on the chemical properties of the blueberry. It was observed that while for moisture content and acidity no significant differences were found among the blueberries from different origins ($p = 0,068$ and $p = 0,051$, respectively) regarding sugars significant differences were encountered ($p = 0.014$), being the blueberries from Estarreja (ES), situated in the North-West of Portugal, the sweetest. Also caliber was found to vary among origins, with highly significant differences ($p < 0.001$), so that the bigger berries were those from Oliveira do Hospital (OH), situated in the North-Center, while the smallest were from Sever do Vouga (SV), situated in the North-West. These observations were made with a 95% confidence.

TABLE I
 KRUSKAL-WALLIS TEST BETWEEN ORIGIN AND THE GENERAL CHEMICAL PROPERTIES AND CALIBER

Property	Origin ¹	N	Mean Rank	χ^2	p-value
Caliber (cm)	SV	30	46.53	43.484	0.000
	ES	30	84.33		
	OH	30	114.63		
	VZ	30	71.97		
	BR	30	60.03		
Moisture (%)	SV	3	10.33	8.728	0.068
	ES	4	5.63		
	OH	3	6.33		
	VZ	4	8.13		
	BR	3	16.00		
Sugars (%)	SV	3	5.00	12.433	0.014
	ES	3	13.33		
	OH	3	2.00		
	VZ	3	11.00		
	BR	3	8.67		
Acidity (mg cit ac/g)	SV	3	8.67	9.428	0.051
	ES	3	3.00		
	OH	3	12.50		
	VZ	3	5.17		
	BR	3	10.67		

¹Locations: SV = Sever do Vouga, ES = Estarreja, OH = Oliveira do Hospital, VZ = Vouzela, BR = Braga.

Gunduz et al. [27] found a significant negative correlation between fruit weight and soluble solids suggesting that as breeders have been selecting for larger fruit, the produced less sweet fruits. In this study the same was observed, since the sample from Oliveira do Hospital showed the highest caliber and the lowest sugar content (Table I).

The results of the Kruskal-Wallis tests between origin and the phenolic properties are shown in Table II. The phenolic compounds: total phenols, anthocyanins or tannins did not evidence, at a level of significance of 5%, statistically

significant differences among origins ($p = 0.117$, $p = 0.816$ and $p = 0.584$, respectively). Regarding the antioxidant activity, depending on the analytical method used, some differences were encountered. In fact the results of the antioxidant activity by ABTS method did not reveal significant differences according to origin ($p = 0.300$), but when determined by FRAP or DPPH methods significant differences were observed ($p = 0.028$ and $p = 0.039$, respectively), and in both cases the berries from Oliveira do Hospital (OH) showed the tendency for the lowest antioxidant activity.

Giovanelli and Buratti [26] reported variation of antioxidant activity of different cultivars of Blueberry, including Bluecrop, in berries from two locations.

TABLE II
KRUSKAL-WALLIS TEST BETWEEN ORIGIN AND THE PHENOLIC PROPERTIES

Property	Origin ¹	N	Mean Rank	χ^2	p-value
Total phenols (mg GAE/g)	SV	24	67.06	7.376	0.117
	ES	24	68.17		
	OH	24	45.67		
	VZ	24	65.79		
	BR	24	55.81		
Anthocyanins (mg Mv3G/g)	SV	24	64.63	1.562	0.816
	ES	24	53.75		
	OH	24	59.96		
	VZ	24	61.38		
	BR	24	62.79		
Tannins (mg/g)	SV	24	60.71	2.847	0.584
	ES	24	62.44		
	OH	24	50.27		
	VZ	24	65.65		
	BR	24	63.44		
ABTS antioxidant activity (mg TE/g)	SV	24	66.98	4.875	0.300
	ES	24	68.83		
	OH	24	51.46		
	VZ	24	61.85		
	BR	24	53.38		
DPPH antioxidant activity (mg TE/g)	SV	24	73.71	10.899	0.028
	ES	24	61.38		
	OH	24	47.58		
	VZ	24	70.08		
	BR	24	49.75		
FRAP antioxidant activity (μ g TE/g)	SV	24	69.52	10.092	0.039
	ES	24	64.29		
	OH	24	43.40		
	VZ	24	70.10		
	BR	24	55.19		

¹Locations: SV = Sever do Vouga, ES = Estarreja, OH = Oliveira do Hospital, VZ = Vouzela, BR = Braga.

Gunduz et al. [27] described significant negative correlations observed between fruit weight and total phenols and FRAP antioxidant activity, which were confirmed in the present work, because the berries from Oliveira do Hospital were the biggest (115 cm) and had lower total phenols content (46 mg GAE/g) and lower FRAP antioxidant activity (43 μ g TE/g).

To test the differences between origins in regards to the physical properties, color and texture, One-Way ANOVA was

used because in this case more experimental determinations were available allowing the application of this parametric test. The results obtained are shown in Table III, and they include the multiple comparisons made with Tukey test. It is possible to verify that for all the physical properties evaluated highly significant differences were found ($p < 0.001$).

Regarding color, the blueberries from Estarreja (ES) and Vouzela (VZ) were lighter, while those from Braga (BR) were darker, with lowest L^* (mean around 31). Also the berries from Braga (BR) had a less intense blue coloration ($b^* = -5$) while those with a stronger blue (mean around -7) were from Estarreja (ES), Oliveira do Hospital (OH) or Vouzela (VZ) (Table III). In these cases the mean values were statistically different from other samples.

As to texture, firmness was higher in sample SV and so was elasticity (mean of 151 N and 2.54 mm, respectively), while the berries from Vouzela (VZ) were less firm (mean of 110 N) and those from Estarreja (ES) were less elastic (mean approximately 2 mm) (Table III). However, in these cases the means were not statistically different from other samples.

TABLE III
ONE-WAY ANOVA BETWEEN ORIGIN AND THE PHYSICAL PROPERTIES

Property	Origin ¹	N	Mean ²	St. dev.	F	p	VE ³				
L^*	SV	57	33.88 ^b	4.18	33.046	.000	33%				
	ES	60	36.38 ^a	2.54							
	OH	52	34.56 ^b	2.00							
	VZ	53	36.79 ^a	3.14							
	BR	58	31.08 ^c	2.67							
	a*	SV	57	0.52 ^a				0.93	9.477	.000	12%
		ES	60	0.09 ^b				0.38			
		OH	52	0.31 ^{ab}				0.43			
		VZ	53	-0.02 ^b				0.46			
		BR	58	0.62 ^a				0.91			
b*	SV	57	-6.19 ^b	1.85	42.653	.000	38%				
	ES	60	-7.77 ^c	1.19							
	OH	52	-7.76 ^c	0.88							
	VZ	53	-7.32 ^c	1.20							
	BR	58	-5.02 ^a	1.51							
Firmness (N)	SV	55	151.33 ^a	26.97	43.224	.000	38%				
	ES	60	119.58 ^b	16.20							
	OH	51	141.14 ^a	20.18							
	VZ	60	110.28 ^b	18.65							
	BR	60	116.01 ^b	17.57							
Elasticity (mm)	SV	55	2.54 ^a	0.53	5.183	.000	7%				
	ES	60	2.18 ^b	0.41							
	OH	51	2.51 ^a	0.42							
	VZ	60	2.41 ^{ab}	0.41							
	BR	60	2.47 ^a	0.63							

¹Locations: SV = Sever do Vouga, ES = Estarreja, OH = Oliveira do Hospital, VZ = Vouzela, BR = Braga.

²Values with the same letter are not statistically different ($\alpha = 5\%$)

³VE: variance explained

B. Effect of Plant Age on the Blueberry Properties

The Pearson coefficient evaluates the strength of the correlations between two quantitative variables. In the present study, the effect of the variable plant age on the physical-chemical properties was assessed through the Pearson coefficients, whose values are presented in Table IV. A very

strong positive association was found between plant age and acidity ($r = 0.736$, correlation significant at the level of 1%), meaning that older blueberry bushes tend to produce fruits with higher acidity. Other significant strong positive correlations was found between plant age and moisture ($r = 0.518$) whereas some significant strong but negative correlations were observed between plant age and sugars ($r = -0.560$) or lightness ($r = -0.534$), so that as the bushes become older, the sugar content tends to diminish and the same happens with L^* , i.e., the fruits tend to be darker. Also some significant but moderate positive correlations were found between age and the opposing color coordinates a^* and b^* ($r = 0.328$ and $r = 0.451$, respectively), and these indicate that older bushes tend to produce berries with higher b^* , i.e., less intense blue color (Table IV).

TABLE IV
PEARSON CORRELATIONS BETWEEN PLANT AGE AND THE PHYSICAL-CHEMICAL PROPERTIES

Property	Pearson correlation coefficient (r)
Caliber	-0.084
Moisture	0.518*
Sugars	-0.560*
Acidity	0.736**
Total phenols	-0.154
Anthocyanins	0.003
Tannins	-0.044
ABTS antioxidant activity	-0.131
FRAP antioxidant activity	-0.166
DPPH antioxidant activity	-0.185*
L^*	-0.534**
a^*	0.328**
b^*	0.451**
Firmness	0.264**
Elasticity	0.190**

*Correlation is significant at the 0.01 level.

**Correlation is significant at the 0.05 level.

C. Effect of Altitude on the Blueberry Properties

Table V shows the Pearson correlation coefficients for the effects of altitude of the plantations in the physical-chemical properties. The results evidence that altitude does not have a significant effect on the majority of the properties studied, having just a strong effect over sugars ($r = 0.622$, correlation significant at the level of 5%) and a moderate effect over firmness ($r = 0.327$, correlation significant at the level of 1%).

D. Effect of Conservation on the Phenolic Properties

To evaluate the differences between the fresh and frozen blueberries on the phenolic properties the independent samples t-test was used because the number of experimental determinations also allowed the application of this parametric test. The results obtained are shown in Table VI, and they reveal that there are no statistically significant differences among the fresh or frozen samples when it comes to total phenols, or antioxidant activity. On the contrary, highly significant differences were encountered in the anthocyanins or tannins contents, being the first higher in the fresh sample (0.19 mg Mv3G/g) whereas the latter were higher in the frozen

sample (1.00 mg/g).

TABLE V
PEARSON CORRELATIONS BETWEEN ALTITUDE AND THE PHYSICAL-CHEMICAL PROPERTIES

Property	Pearson correlation coefficient (r)
Caliber	0.099
Moisture	-0.284
Sugars	0.622*
Acidity	0.262
Total phenols	-0.061
Anthocyanins	-0.044
Tannins	-0.036
ABTS antioxidant activity	-0.019
FRAP antioxidant activity	0.054
DPPH antioxidant activity	0.004
L^*	0.190**
a^*	-0.061
b^*	-0.226**
Firmness	0.327**
Elasticity	0.151*

*Correlation is significant at the 0.01 level.

**Correlation is significant at the 0.05 level.

TABLE VI
INDEPENDENT SAMPLES T-TEST BETWEEN CONSERVATION AND THE PHENOLIC PROPERTIES

Property	Conservation ¹	N	Mean	sd ²	Levene ³	t	p
Total phenols	Fresh	60	1.16	1.05	0.866	0.273	0.393
	Frozen	60	1.11	1.06			
Anthocyanins	Fresh	60	0.19	0.29	0.000	5.079	0.000
	Frozen	60	0.00	0.00			
Tannins	Fresh	60	0.65	0.52	0.098	-2.584	0.005
	Frozen	60	1.00	0.88			
ABTS ant. activity	Fresh	60	0.87	0.81	0.001	1.445	0.076
	Frozen	60	1.15	1.26			
DPPH ant. activity	Fresh	60	0.62	0.64	0.032	-2.009	0.024
	Frozen	60	0.87	0.79			
FRAP ant. activity	Fresh	60	15.03	14.53	0.140	0.949	0.173
	Frozen	60	12.73	11.95			

¹Frozen: during 6 months.

²sd: standard deviation.

³Significance of the Levene test for equality of variances.

E. Effect of Extraction on the Phenolic Properties

Table VII shows the results of the independent samples t-test for the effect of the extraction solvent on the phenolic contents and antioxidant activity. From the results obtained it was concluded that the type of solvent used had a marked influence on the properties evaluated, because in all cases statistically significant differences were observed in the methanol and acetone extracts in terms of total phenols ($p < 0.001$), anthocyanins ($p < 0.001$), tannins ($p = 0.002$) or ABTS ($p < 0.001$), DPPH ($p < 0.001$) and FRAP ($p < 0.001$) antioxidant activities. Furthermore, the results in Table VII indicated that total phenols were extracted in a higher quantity by methanol when compared to acetone. Also for anthocyanins a similar trend was observed. Reading tannins, however, a contradictory trend was observed, so that these were better extracted by acetone. As to the antioxidant activity, for all method used the values observed were higher

in the methanol extracts as compared to the acetone extracts.

TABLE VII
INDEPENDENT SAMPLES T-TEST BETWEEN EXTRACTION SOLVENT AND THE PHENOLIC PROPERTIES

Property	Solvent	N	Mean	sd ¹	Levene ²	t	p
Total phenols	Methanol	60	1.62	1.29	0.000	5.689	0.000
	Acetone	60	0.65	0.30			
Anthocyanins	Methanol	60	0.19	0.29	0.000	4.735	0.000
	Acetone	60	0.01	0.01			
Tannins	Methanol	60	0.63	0.44	0.000	-2.922	0.002
	Acetone	60	1.02	0.92			
ABTS ant. activity	Methanol	60	1.50	1.29	0.000	5.728	0.000
	Acetone	60	0.51	0.37			
DPPH ant. activity	Methanol	60	1.02	0.90	0.000	5.077	0.000
	Acetone	60	0.46	0.32			
FRAP ant. activity	Methanol	60	19.49	15.91	0.000	4.483	0.000
	Acetone	60	8.27	6.29			

¹sd: standard deviation.

²Significance of the Levene test for equality of variances.

TABLE VIII
ONE-WAY ANOVA BETWEEN EXTRACTION ORDER AND THE PHENOLIC PROPERTIES

Property	Order	N	Mean ¹	St. dev.	F	p	VE ²
Total phenols	1 st	30	2.76 ^a	0.82	209.609	0.000	84%
	2 nd	30	0.48 ^c	0.10			
	3 rd	30	0.93 ^b	0.16			
	4 th	30	0.37 ^c	0.04			
Anthocyanins	1 st	30	0.34 ^a	0.36	23.769	0.000	38%
	2 nd	30	0.04 ^b	0.04			
	3 rd	30	0.01 ^b	0.01			
	4 th	30	0.00 ^b	0.00			
Tannins	1 st	30	0.95 ^a	0.32	44.965	0.000	54%
	2 nd	30	0.31 ^c	0.27			
	3 rd	30	1.66 ^b	0.89			
	4 th	30	0.38 ^c	0.28			
ABTS	1 st	30	2.65 ^a	0.80	228.463	0.000	86%
	2 nd	30	0.36 ^c	0.10			
	3 rd	30	0.86 ^b	0.15			
	4 th	30	0.16 ^c	0.07			
DPPH	1 st	30	1.79 ^a	0.64	139.866	0.000	78%
	2 nd	30	0.25 ^c	0.11			
	3 rd	30	0.74 ^b	0.22			
	4 th	30	0.19 ^c	0.07			
FRAP	1 st	30	33.78 ^a	9.36	217.051	0.000	85%
	2 nd	30	5.19 ^c	2.21			
	3 rd	30	13.80 ^b	3.77			
	4 th	30	2.75 ^c	1.72			

¹Values with the same letter are not statistically different ($\alpha = 5\%$)

²VE: variance explained

Table VIII presents the results of the Anova test for comparison of the phenolic properties in multiple groups, in this case, the extraction order, with multiple comparisons by Tukey test. In all cases highly significant differences were observed ($p < 0.001$) and the results of the multiple comparison show that the first (methanol) extract always shows higher values than the other extracts, being these differences statistically significant. The extract which follows is the third (the first acetone extract), showing significant

differences from the others in most cases.

F. Correlations between the Physical-Chemical Properties

The Pearson correlation coefficients between some chemical properties are shown in Table IX and they reveal that there is only one value which is statistically significant, the correlation between sugars and acidity, which is strong and negative ($r = -0.658$), indicating that as the sugar content increases the acidity diminishes.

TABLE IX
PEARSON CORRELATIONS BETWEEN THE GENERAL CHEMICAL PROPERTIES

	Caliber	Moisture	Sugars	Acidity
Caliber	1			
Moisture	-0.429	1		
Sugars	-0.156	0.130	1	
Acidity	0.060	0.381	-0.658**	1

**Correlation is significant at the 0.01 level.

Table X shows the Pearson correlation coefficients between the physical properties. Only one very strong correlation was found, between blueness (b^*) and lightness (L^*) ($r = -0.911$), indicating that the berries with higher L^* had lower b^* , i.e., the berries that were not so dark were more blue. Then, moderate correlations were found between a^* and L^* ($r = -0.463$) and between a^* and b^* ($r = 0.442$), being however negative in the first case and positive in the second. Also firmness was found positively moderately correlated with elasticity, so that the berries with higher firmness also presented higher elasticity.

TABLE X
PEARSON CORRELATIONS BETWEEN THE PHYSICAL PROPERTIES

	L^*	a^*	b^*	Firmness	Elasticity
L^*	1				
a^*	-0.463**	1			
b^*	-0.911**	0.442**	1		
Firmness	-0.124*	0.146*	0.044	1	
Elasticity	-0.208**	0.117	0.182**	0.404**	1

**Correlation is significant at the 0.01 level.

*Correlation is significant at the 0.05 level.

The correlation coefficients between the phenolic properties are presented in Table XI. Very strong correlations were found between total phenols and antioxidant activity, regardless of the determination method used (0.919, 0.939 and 0.911 for ABTS, DPPH and FRAP methods), all significant at the level of 1%. Also a very strong correlation was encountered between ABTS and DPPH ($r = 0.902$) or between FRAP and DPPH ($r = 0.961$) or even between FRAP and DPPH ($r = 0.905$) at a level of significance of 1%.

The results in Table XI further demonstrate that total phenols and anthocyanins are positively strong correlated ($r = 0.576$), indicating that samples with higher total phenols also possessed higher anthocyanins contents. As to tannins content, it was found moderately correlated with DPPH ($r = 0.329$) and FRAP ($r = 0.355$) antioxidant activities.

TABLE XI
PEARSON CORRELATIONS BETWEEN THE PHENOLIC PROPERTIES

	TP	ANT	TAN	ABTS	DPPH	FRAP
TP	1					
ANT	0.576**	1				
TAN	0.246**	0.144	1			
ABTS	0.919**	0.390**	0.259**	1		
DPPH	0.939**	0.658**	0.329**	0.902**	1	
FRAP	0.911**	0.389**	0.355**	0.961**	0.905**	1

**Correlation is significant at the 0.01 level.

*Correlation is significant at the 0.05 level.

IV. CONCLUSION

The results obtained in this study allowed identifying some differences among the samples according to different factors. The origin of the blueberries showed influence on the sugar content and on the size, on the antioxidant activity when measured by FRAP and DPPH methods, and on all color and texture parameters. The conservation had influence on the anthocyanins and DPPH antioxidant activity only. On the contrary, extraction solvent and extraction order showed a very pronounced influence on the phenolics contents and antioxidant activity.

Very strong correlations were found between total phenols and antioxidant activity, regardless of the method used for the determination, or even between the different antioxidant activity determinations. Also a very strong correlation was found between lightness and blueness.

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REFERENCES

- Q. You, B. Wang, F. Chen, Z. Huang, X. Wang, and P. G. Luo, "Comparison of anthocyanins and phenolics in organically and conventionally grown blueberries in selected cultivars," *Food Chemistry*, vol. 125, no. 1, pp. 201–208, Mar. 2011.
- A. D. R. Castrejón, I. Eichholz, S. Rohn, L. W. Kroh, and S. Huyskens-Keil, "Phenolic profile and antioxidant activity of highbush blueberry (*Vaccinium corymbosum* L.) during fruit maturation and ripening," *Food Chemistry*, vol. 109, no. 3, pp. 564–572, Aug. 2008.
- C. Contessa, M. G. Mellano, G. L. Beccaro, A. Giusiano, and R. Botta, "Total antioxidant capacity and total phenolic and anthocyanin contents in fruit species grown in Northwest Italy," *Scientia Horticulturae*, vol. 160, pp. 351–357, Aug. 2013.
- Q. Zhou, C. Zhang, S. Cheng, B. Wei, X. Liu, and S. Ji, "Changes in energy metabolism accompanying pitting in blueberries stored at low temperature," *Food Chemistry*, vol. 164, pp. 493–501, Dec. 2014.
- K. A. Youdim, B. Shukitt-Hale, A. Martin, H. Wang, N. Denisova, P. C. Bickford, and J. A. Joseph, "Short-Term Dietary Supplementation of Blueberry Polyphenolics: Beneficial Effects on Aging Brain Performance and Peripheral Tissue Function," *Nutritional Neuroscience*, vol. 3, no. 6, pp. 383–397, 2000.
- S. Y. Wang, M. J. Camp, and M. K. Ehlenfeldt, "Antioxidant capacity and α -glucosidase inhibitory activity in peel and flesh of blueberry (*Vaccinium* spp.) cultivars," *Food Chemistry*, vol. 132, pp. 1759–1768, Jun. 2012.
- G. D. Stoner, L.-S. Wang, and B. C. Casto, "Laboratory and clinical studies of cancer chemoprevention by antioxidants in berries," *Carcinogenesis*, vol. 29, no. 9, pp. 1665–1674, Sep. 2008.
- R. Zadernowski, M. Naczka, and J. Nesterowicz, "Phenolic Acid Profiles in Some Small Berries," *J. Agric. Food Chem.*, vol. 53, no. 6, pp. 2118–2124, Mar. 2005.

- A. Howell, W. Kalt, J. C. Duy, C. F. Forney, and J. E. McDonald, "Horticultural Factors Affecting Antioxidant Capacity of Blueberries and other Small Fruit," *HortTechnology*, vol. 11, no. 4, pp. 523–528, Jan. 2001.
- G. Giovanelli and S. Buratti, "Comparison of polyphenolic composition and antioxidant activity of wild Italian blueberries and some cultivated varieties," *Food Chemistry*, vol. 112, pp. 903–908, Feb. 2009.
- W. Kalt, "Effects of Production and Processing Factors on Major Fruit and Vegetable Antioxidants," *Journal of food science*, vol. 70, no. 1, 2005.
- W. Kalt, J. e. McDonald, and H. Donner, "Anthocyanins, Phenolics, and Antioxidant Capacity of Processed Lowbush Blueberry Products," *Journal of Food Science*, vol. 65, no. 3, pp. 390–393, Apr. 2000.
- L. Gao and G. Mazza, "Quantitation and Distribution of Simple and Acylated Anthocyanins and Other Phenolics in Blueberries," *Journal of Food Science*, vol. 59, no. 5, pp. 1057–1059, Sep. 1994.
- H. Böhm, "G. Mazza und E. Miniati: Anthocyanins in Fruits, Vegetables and Grains. 362 Seiten, zahlr. Abb. und Tab. CRC Press, Boca Raton, Ann Arbor, London, Tokyo 1993. Preis: 144.— £," *Nahrung*, vol. 38, no. 3, pp. 343–343, Jan. 1994.
- M. Sousa, T. Curado, and S. Vieira, "Características físicas, químicas e sensoriais de cultivares de mirtilo (*Vaccinium* sp) introduzidas em Portugal," *Atas 5º Encontro de Química de Alimentos: Qualidade, Segurança, Inovação*, vol. 1, pp. 349–351, 2001.
- F. J. Gonçalves, S. M. Rocha, and M. A. Coimbra, "Study of the retention capacity of anthocyanins by wine polymeric material," *Food Chem*, vol. 134, no. 2, pp. 957–963, Sep. 2012.
- R. Boulton, "The Copigmentation of Anthocyanins and Its Role in the Color of Red Wine: A Critical Review," *Am. J. Enol. Vitic.*, vol. 52, no. 2, pp. 67–87, Jan. 2001.
- P. Ribereau-Gayon and E. Stonestreet, "Dosage des tanins du vin rouge et détermination de leur structure," *Chimie Anal*, vol. 48, pp. 188–196, 1966.
- W. Brand-Williams, M. E. Cuvelier, and C. Berset, "Use of a free radical method to evaluate antioxidant activity," *LWT - Food Science and Technology*, vol. 28, no. 1, pp. 25–30, 1995.
- N. J. Miller, C. Rice-Evans, M. J. Davies, V. Gopinathan, and A. Milner, "A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates," *Clin. Sci.*, vol. 84, no. 4, pp. 407–412, Apr. 1993.
- I. F. Benzie and J. J. Strain, "The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay," *Analytical biochemistry*, vol. 239, no. 1, pp. 70–6, Jul. 1996.
- N. Deighton, R. Brennan, C. Finn, and H. V Davies, "Antioxidant properties of domesticated and wild *Rubus* species," *Journal of the Science of Food and Agriculture*, vol. 80, no. January, pp. 1307–1313, 2000.
- J. Maroco, *Análise Estatística com o SPSS Statistics*, 5ª Ed. Brazil: Report number, 2012.
- M. H. Pestana and J. N. Gageiro, *Análise de Dados para Ciências Sociais – A complementaridade do SPSS*, 6ª ed. Brasil: Edições Sílabo, 2014.
- J. P. Stevens, *Applied Multivariate Statistics for the Social Sciences, Fifth Edition*, 5 edition. New York: Routledge, 2009.
- G. Giovanelli and S. Buratti, "Comparison of polyphenolic composition and antioxidant activity of wild Italian blueberries and some cultivated varieties," *Food Chemistry*, vol. 112, no. 4, pp. 903–908, Feb. 2009.
- K. Gündüz, S. Serçe, and J. F. Hancock, "Variation among highbush and rabbiteye cultivars of blueberry for fruit quality and phytochemical characteristics," *Journal of Food Composition and Analysis*, vol. 38, pp. 69–79, Mar. 2015.