DETERMINATION OF CHROMATIC CHARACTERISTICS OF THE HYDROALCOHOLIC EXTRACTS OBTAINED FROM THE FRUITS OF SOME CHERRY AND SOUR CHERRY VARIETIES

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Abstract

Data on chromatic parameters allow objective assessment of fruit color at maturity of consumption, by analyzing hydroalcoholic extracts obtained and monitoring color development during their storage. This paper aims to determine the chromatic parameters (L, a, b, C, H°) and anthocyanin content of ethanolic extracts obtained from fruits of four varieties of sour cherry (*Prunus cerasus* L.) and six varieties of cherry (*Prunus avium* L .) grown in the experimental field of Research Development Station for Fruit growing, Iasi, using CIE Lab-76 method and data digitization program *Vincolor* modified. Bitter cherry variety Amar de Maxut, presented the most important content in anthocyanins (443.72±1.54 mg/100g) and total phenolic compounds (794.62±0.08 mg GAE/100g), this variety having the most intensely colored fruits of the analyzed varieties. The color of each extract was simulated electronically using the program Digital Colour Atlas® 3.0 Demoversion, based on values obtained from spectral analysis (λ 380-780 nm).

Key words: chromatic parameters, cherry, sour cherry, anthocyanins

In the choice and consumption of food, color plays an important role, along with taste, beeing associated with food quality and safety (Beceanu D. *et al.,* 2011). The consumer recognizes the color as a primary attribute of a food product (Socaciu Carmen, 2008). It is therefore necessary to know the specific color parameters for each source of pigments used in food industry. A current problem is to identify new sources of plant pigments, to replace synthetic dyes currently used widely in the food industry (Davies K., 2004). Anthocyanins in cherries and cherry extracts are currently used for this purpose (E163), together with those from other plant sources. The color of extracts can be identified based on their color characteristics and can be influenced by the chemical composition of vegetal sources. CIE Lab-76 model, proposed by the *Commission Internationale pour Eclairage* (CIE) allows an objective and complete evaluation of chromatic characteristics of the samples analyzed.

MATERIAL AND METHOD

Were determined the color characteristics of hydroalcoholic extracts obtained from fruits of four varieties of sour cherry (*Prunus cerasus L.*): Crişane 2, Engleze timpurii, Meteor korai şi Mocăneşti 16 and six varieties of cherry (*Prunus avium L.*): Amar de Maxut, Maria, Oana (*HC-840860*), Radu (*HC-840836*), Stella şi Van, grown in the experimental field of the Research Development Station for Fruit growing (RDSF), Iasi. Fruits were harvested at maturity of consumption when they realized the characteristic attributes of the variety (Grădinariu G. *et al.*, 1998). To determine the degree of maturity at wich the fruits have been harvested and for more precise identification of that moment, were registered several phenological data, at the RDSF Iasi (flowering date, number of days from flowering to harvest, harvest date). Until determinations, the fruits were kept frozen (-18°C±2) transformation of anthocyanins being considered as minimal (Mazza G., Miniati E., 1993). Extraction system used was ethanol-HCl-water by reason of food purpose of the extracts (96:1:3) (pH 1.5±0.1) (Filimon R. *et al.*, 2011). An ultrasound treatment was applied on the samples to reduce aggregation and agglomeration of particles. The three extraction fractions obtained were cumulated. To determine total monomeric anthocyanins, was used the spectrophotometric pH differential method. In acidic environment, there is a balance between colored and colorless forms of anthocyanins. This balance is a function of pH (Lee J. *et al.*, 2008). Coloring intensity variation between two pH values (0.68 and 3.56), determined at a wavelength (λ) 520 nm, is proportional to the total amount of monomeric anthocyanins (mg anthocyanins/100 g fresh fruit) (Compendium of international Methods OIV, 2008). To obtain the total phenolic content was used Folin-

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Ciocalteu colorimetric method (IFC) at λ 765 nm, results were expressed in gallic acid equivalent (mg GAE)/100g fresh fruit (Compendium of International Methods OIV, 2008).

We determined several physicochemical properties of the fruits: the average fruit weight, moisture content (drying oven at 105°C, 4 hours), total dry substance, soluble solids (refractometry), titratable acidity (titration method with NaOH), reducing sugars (Schoorl method), pH of the juice (potentiometric method), as influencing factors on anthocyanin pigment content (from skin and pulp).

The CIE L* a* b* model is the most complete color space specified by CIE (*fig. 1*), created to serve as an independent device, a reference model. In the CIE system, a color can be plotted in a three-dimensional space through the coordinate points (X, Y, Z). All points corresponding to all possible colors, forms the color space.

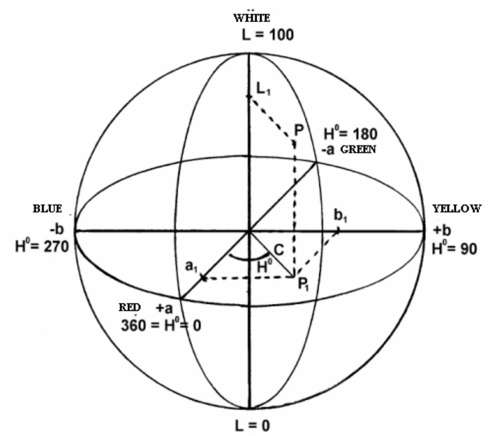

Figure 1 **CIE linear color space Lab - 76 and LCH°**

The measurement of colors in CIE system consists in determining the values of *L* (brightness coordinate or psychrometric clarity), *a* (red-green coordinate) and b (yellow-blue coordinate) to which are added parameters: C (chromaticity or saturation), H° (tone), I (intensity, $A_{420}+A_{520}+A_{620}$) and H (color hue). Chromatic components were calculated based on absorbance spectra recorded in the visible domain (VIS), within the wavelength (λ) 380-780 nm, with an Analytik Jena S-200 spectrophotometer, coupled with a computer, using a glass cuvette with 1 cm optical path. Was made digitization and automatic recording of absorption spectra in a file using the "*Vincolor*" improved software, to obtain the chromatic parameters (L*,a*,b*, C, H°) (Coşofreţ S. *et al.*, 1997).

CIE called measurable distance between two color ΔE* ab, where delta (Δ) is used to note the differences, and E is the notion of sensation (Zamfir, C., 2009). Difference between two colors or ΔE is given by the radical (√) of the formula: $\Delta E = (L_1-L_2)^2+(a_1-a_2)^2+(b_1-b_2)^2$. Was used for comparison the extract that showed the highest values of the parameters. Determination of chromatic characteristics (color intensity and hue) was performed according to usual methods of OIV.

Color of each extract was simulated using Digital Colour Atlas® 3.0 Demo version software, based on chromatic parameters, to reflect the sensorial differences between extracts color.

RESULTS AND DISCUSSIONS

For more precise determination of harvest time (maturity of consumption) was recorded several phenological data of the varieties (tab. 1).

Table 1
Phenological data of the analyzed varieties

No.	Variety	End flow.*	Date mat.*	No. days fl.-rip.*
1	Engleze timpurii	15.04	12.06	59
2	Crişane 2	15.04	20.06	67
3	Mocăneşti 16	15.04	22.06	69
4	Meteor korai	17.04	12.06	57
5	Oana	10.04	07.06	59
6	Radu	10.04	08.06	60
7	Maria	17.04	16.06	61
8	Van	19. 04	15.06	58
9	Stella	20.04	16.06	58
10	Amar de Maxut	20.04	22.06	64

** End flow. – End of flowering; Date mat. – Date of fruit maturation; No. days fl.-rip. – Number of days from flowering to ripening*

The minimum number of days from flowering to ripening and harvest, was minimal at sour cherry variety Meteor korai (57 days) and at cherry varieties Stella and Van (58 days).

Physicochemical properties of the fruits (*tab. 2*) had specific values for each variety. Thus, at sour cherry varieties, Crişane 2 average fruit weight was highest (6.2 g) and at the variety Engleze timpurii, soluble solids content (16.52°Bx), total dry matter (18.19 %) and pH (3.62) values, were most important among studied varieties, this being a variety obtained by interspecific hybridization between sweet cherry and sour cherry. At cherry variety Van, soluble sugars content was the most significant (10.42 mg glucose/100g) and Radu variety has the highest pH (3.9), titratable acid content (3.97 g malic acid/ 100 g) and total dry substance (20.36%).

After the interpretation of absorption spectra at wavelengths specific to each class of compounds, results were achieved for total anthocyanins and total phenolic content (*tab. 3*). Obtained data represent the average of three determinations and have calculated the standard deviation. Variety of bitter cherry fruit Amar de Maxut, presented the most important content in anthocyanins (443.72±1.54 mg/100g) and phenolic compounds (794.62±0.08 mg GAE/100g), this variety having the most intensely colored fruits of varieties selected for study. Among the sour cherry varieties, Engleze timpurii held the maximum amount of anthocyanin pigment (176.20± 0.97 mg/100 g) and variety Mocăneşti 16, of total phenolic compounds (446.89±0.70 mg GAE /100g).

Absorbance spectra recorded in 380-780 nm wavelength range of sour cherry (fig. 2) and cherry samples (fig. 3) were recorded in the program *Vincolor* (fig. 3), resulting the colour parameters for each extract.

Table 2

The physico-chemical properties of sour cherry and cherry fruits studied

No.	Variety	M. fr.* (g)	M.* (%)	T. ac.* (g m.a.)	Rd. Sg.* (mg glucose)	pH (units)	TDS* (%)	SDS* (°Bx)
1	Engleze timpurii	4,9	81,81	0,73	5,70	3,62	18,19	16,52
2	Meteor Korai	5,3	85,87	0,80	7,81	3,31	14,13	13,80
3	Mocăneşti 16	4,8	87,98	1,32	7,36	3,24	12,02	10,92
4	Crişana 2	6,2	84,89	1,28	6,78	3,27	15,11	14,70
5	Van	6,5	84,22	0,72	10,42	3,65	15,78	14,12
6	Stella	6,6	86,41	0,36	5,97	3,83	13,59	12,72
7	Maria	7,3	83.92	0,44	9,15	3,69	16,08	14,62
8	Radu	6,4	79,64	0,73	7,22	3,97	20,36	17,12
9	Oana	7,4	81,72	0,44	8,18	3,91	18,28	16,72
10	Amar Maxut	4,2	81,78	0,73	8,46	3,59	18,22	14,72

* M. fr. – average mass of fruit; M (%) – moisture; T. ac. (g m. a.) – titratable acidity (g malic acid /100g); Rd. sg. (g. %) – reducing sugars (mg glucose/100 g FW); SDS (°Bx) – soluble dry substance (°Brix); TDS (%) – total dry substance.

Table 3

CA (anthocyanin content) and TPC (total phenolic compounds) values of the studied samples

No.	Variety	CA (mg/100g)	TPC (mg GAE/100g)
1	Mocăneşti 16	107,46±0,29	446,89±0,70
2	Crişana 2	117,12±0,91	370,18±0,19
3	Meteor Korai	117,50±0,99	321,43±0,14
4	Engleze timpurii	176,20±0,97	325,18±0,14
5	Van	46,84±0,88	162,62±0,33
6	Stella	77,34±0,45	240,46±0,26
7	Maria	154,19±0,90	352,17±0,21
8	Radu	191,26±0,64	387,56±0,19
9	Oana	378,93±1,02	563,42±0,42
10	Amar Maxut	443,72±1,54	794,62±0,08

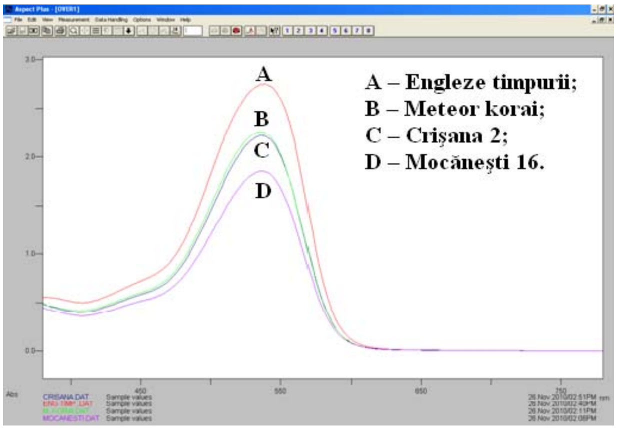

Figure 2 **Overlay of the absorption spectra at sour cherry varieties**

By analyzing the data and spectra obtained can be observed that the varieties with a high content of anthocyanins, had the most significant increase of the spectral line at wavelength 520 nm, specific to these phenolic compounds, confirming anthocyanins participation in fruit color formation, as the main constituents.

From the analysis of the *L* parameter (brightness) (*tab. 3*), resulted that extracts of sour cherry presented psychrometric clarity, values were very similar, ranging between 53.6 (Engleze timpurii) and 59.3 (Mocăneşti 16).

The cherry extracts clarity *(tab. 4)* had values that varies much larger, from 42.1 (Amar de Maxut) to 78.4 (Van), this fact results also from the visual inspection of computer simulated colors, obtained using parameters determined previously.

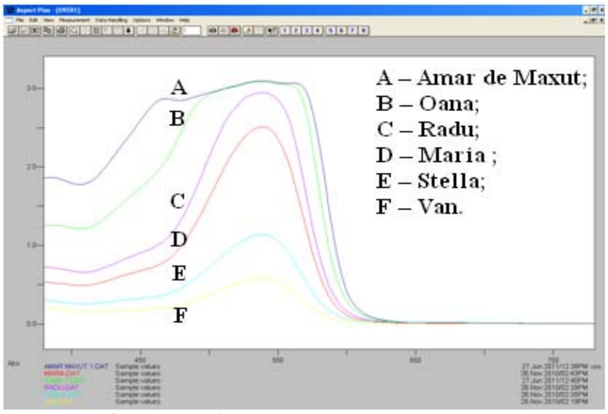

Figure 3 **Overlay of the absorption spectra at cherry varieties**

All extracts presented coordinate of complementary color *red* (due to high content in anthocyanins) and *yellow*, except extracts of varieties of cherry Stella (-11.80) and Van (-9.91) and the variety of sour cherry Mocăneşti 16 (-2.82), with negative values of the *b* parameter, indicating the presence of complementary color *blue* in the color composition of hydroalcoholic extracts. Other chromatic parameters C and H° results from the transformation of orthogonal cartesian coordinates *a* and *b*, in polar coordinates. *L* coordinate remains the same in both systems of representation: saturation $C = (a^2 + b^2)^{1/2}$ and color hue (tint) H° = arctg (b/a).

At sour cherries, color saturation values (C) were maintained in small limits of variation from variety to variety (75.49 to 80.54), which also results from very close shades of samples analyzed color (tab. 4). With the increase in C parameter, changes occurs in the color visually inspected, so cherry variety Amar de Maxut, which has the highest saturation value (100.36), also had the darkest color of the fruit and extract (tab. 5).

Correlation between values of parameter H° and colors perceived visually is part of the linear color space CIE-LCH°. Tonality of color had negative values at cherry varieties Stella (-10.57) and Van (-13.20) and at the variety of sour cherry Mocăneşti 16 (-2.14), establishing a direct correlation between the parameter H° and *b* coordinate (yellow-blue), with negative values of these features at the same varieties. Considering that the tint angle H° = 90° corresponds to color *yellow* (Odăgeriu G. *et al.*, 2005), we see that in relation to this reference, is understandable that the samples with H° less than 90°, visually are perceived with certain nuances of blue. Colour intensity (I), had very close values at the varieties of sour cherries (from 2.07 to 3.06), while at cherries extracts this parameter varies from 0.67, at Van variety, up to 5.01 at Amar de Maxut variety. Clear - *red* hue is even more pronounced as more hue angle is close to zero and therefore is less influenced by yellow hues (Odăgeriu G. *et al.*, 2008).

At sour cherries, the parameter H is very close to zero (*tab. 3*), three of the four varieties examined reaching to 0.21. Among cherry extracts, only variety Amar de Maxut, with fruit colored in dark red to black, cromathic feature H reached 0.63 (tab. 4).

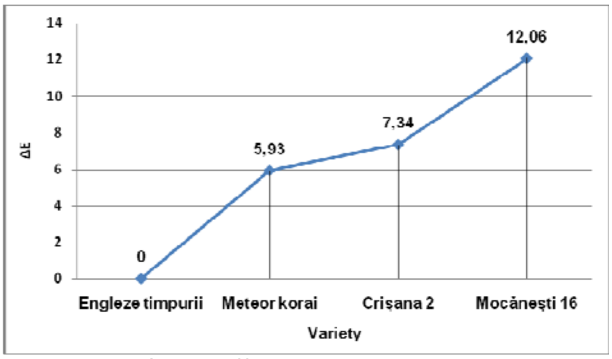

Figure 4 **Color differences (ΔE) at sour cherry varieties**

Color differences (ΔE) between fruit extracts were obtained by applying the formula. Theoretically, in a ΔE <1.0, between two colors is assumed that the difference would be sensorial imperceptible (Zamfir C., 2009). At sour cherries, the differences were calculated depending on the Engleze timpurii variety, as reference (fig. 4), with the most important value of the parameter ΔE at the Mocăneşti 16 variety (12.06). At cherries, differences between chromatic parameters of the variety Amar de Maxut, as reference, and the other varieties analyzed, showed the greatest contrast of color, expressed by the value of ΔE, at Van variety (90.56) (*fig. 6*). More important color differences existing at the varieties of cherries, in comparison with those of sour cherry can be noticed visually by analyzing the simulated colors.

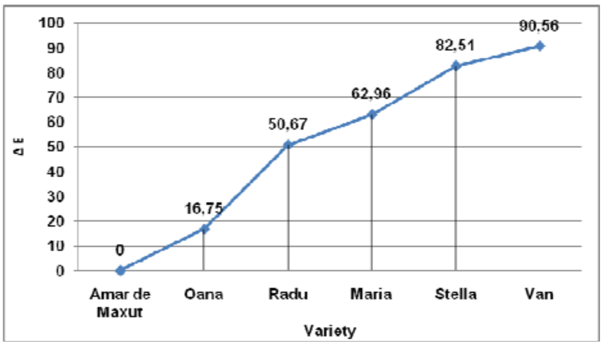

Figure 5 **Color differences (ΔE) at cherry varieties**

Visually perceived color of the extracts is found in a limited color palette: light red, red - brown, reddish - brown, covering a part of the visible spectrum.

A computerised simulation was made to the color of each extract, using the software Digital Colour Atlas® 3.0 Demoversion (fig. 6), based on the chromatic parameters previously calculated, to highlight differences in color and classifying the extracts via sensorial way (visual).

Between physicochemical characteristics investigated and chromatic parameters of cherries and sour cherries extracts could not be established a significant positive correlation. It was analyzed the possibility of correlations between chromatic parameters calculated and the amount of anthocyanins and total polyphenols. using the program Microsoft Excel®, Data Analysis Tools.

Table 3

Chromatic parameters of the sour cherry fruit extracts

No.	Variety	Color simulation	L*	Color coordinates		C	H°	H	I
				a*	b*				
1	Engleze timpurii		53,6	80,26	6,66	80,54	4,74	0,21	2,46
2	Meteor korai		57,1	78,24	2,32	78,27	1,70	0,21	3,06
3	Crişana 2		57,2	78,37	0,55	78,37	0,40	0,21	2,53
4	Mocăneşti 16		59,3	75,44	-2,82	75,49	-2,14	0,23	2,07

* L – brightness (clarity): 0 (opaque) - 100 (transparently); a – red (+) - green (-); b – yellow (+) - blue (-); C – saturation; H° – tone; H – color tint; I – intensity of color.

Table 4

Chromatic parameters of the cherry fruit extracts

No.	Variety	Color simulation	L*	Color coordinates		C	H°	H	I
				a*	b*				
1	Amar de Maxut		42,1	75,49	66,12	100,36	41,21	0,63	5,01
2	Oana		44,8	76,61	49,62	91,28	32,92	0,42	4,36
3	Radu		50,8	79,08	16,33	80,75	11,66	0,24	3,50
4	Maria		54,5	78,95	4,49	79,08	3,25	0,23	2,79
5	Stella		66,3	63,22	-11,80	64,32	-10,57	0,25	1,29
6	Van		78,4	42,28	-9,91	43,43	-13,20	0,29	0,67

* L – brightness (clarity): 0 (opaque) - 100 (transparently); a – red (+) - green (-); b – yellow (+) - blue (-);
C – saturation; H° – tone; H – color tint; I – intensity of color.

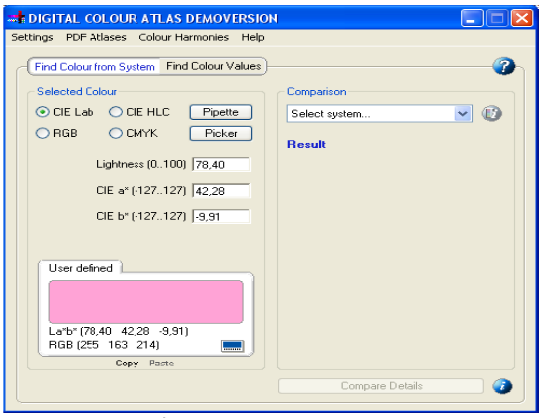

Figure 6 **Aspect from the colors simulation program**

Was found a major positive correlation (R^2=0.98) between anthocyanin content and *b* parameter (*fig. 8*), meaning that a low content in anthocyanins pigments makes the parameter *b* values to evolve negatively, as happened at cherry varieties Stella (-11.80) and Van (-9.91), and at the sour cherry variety Mocăneşti 16 (-2.82), indicating the appearance of complementary color blue in the chromatic composition of the extracts.

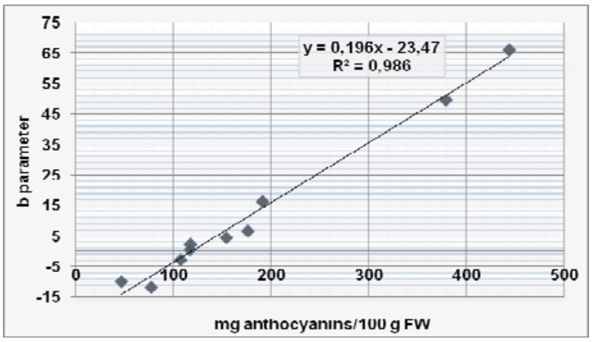

Figure 8. **Correlation between anthocyanin content and chromatic parameter *b***

Was determined a direct relation (R^2=0.98) between color tone (H°) and anthocyanin content of fruits (*fig. 9*). Correlation between H° parameter and colors perceived visually, is included in the plan a_1Ob_1 (*fig. 1*), as follows: red 0°, yellow 90°, green 180°. Low or even negative values of tonality, recorded at some varieties of sour cherries (Mocăneşti 16: -2.14) and cherries (Van: -13.20; Stella: -10.57), indicates the position of the parameter near to the 0° angle, corresponding to color *red*, based on the presence of anthocyanins.

Between total phenolics content and the two parameters correlated above with the presence of anthocyanins (*b* and H°), was found the same positive relationship. This can be simply explained by the fact that anthocyanins are part of the phenolic compounds. In cherries, anthocyanins are the second largest class of phenolic compounds (after hydroxycinnamates), with a rate of about 26% of total phenolics (Kelley D.S. et al., 2006).

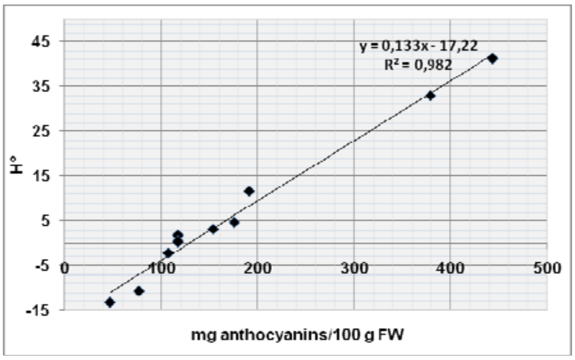

Figure 9. **Correlation between anthocyanin content and chromatic parameter H°**

All correlations had calculated a probability *p-value* < 0.01, determined by the Analysis of variance (ANOVA test, single factor), indicating that the results are statistically significant.

CONCLUSIONS

The four varieties of sour cherries and six varieties of cherries analyzed, showed a number of physical and chemical characteristics, with specific values. Total anthocyanin content had the highest value in the sour cherries, at Engleze timpurii variety (176.2±0.97 mg/100g) and in cherries, at variety Amar de Maxut (443.72±1.54 mg /100g), this having the most intensely colored fruits.

It should be noted that at cherries exist a positive correlation between anthocyanin and total phenolic content, which at sour cherries could not be demonstrated, ratio between anthocyanins and phenolic compounds was in inverse proportion that in cherries.

Data concerning color features (chromatic parameters, intensity and hue), determined for each sample, allowing an objective assessment of extracts color, on their quality and traceability in the evolutive phenomena, mainly related to the structural and biochemical changes in anthocyanins, the principal participants in the formation of fruits red color.

Recording of the chromatic parameters and their storage in databases, allowing anytime the electronic simulation of the initial color of extracts or fruit juices, with the possibility of visual comparison, if the aims of anthocyanin extracts is use in the food or pharmaceutical sector, and also in the experiences which determined the evolution in time of the chromatic characteristics. Determining the chromatic parameters helps to highlight differences in fruits color between varieties from the same species or between the same varieties from different growing areas.

The order of varieties, resulting from the anthocyanins amount (*tab. 3*), is the same with the positioning of absorption spectra in spectrogrames, and the visual observed color.

Major positive correlations were found (R^2=0.98, $p<0.01$) between anthocyanin content and some color parameters (*b* and H°), confirming that these phenolic compounds are the major constituents of color, in analyzed fruits.

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