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ORIGINAL RESEARCH PAPER

THE INFLUENCE OF TEMPERATURE AND TIME ON THE ANTIOXIDANT ACTIVITY AND COLOR PARAMETERS OF DOG-ROSE (*ROSA CANINA*) ETHANOLIC EXTRACT

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Abstract: This paper presents the study of the stability of antioxidant activity and color of the 50 % ethanolic extract of dog-rose (*Rosa canina*) from the Republic of Moldova with the main objective to test a food dye of natural origin. The extract was submitted to: -2 °C for 12 hours; 4 °C for 12 hours; 40 °C for 15 minutes, 60 °C for 15 minutes, 80 °C for 15 minutes and 100 °C for 2 minutes, after which the antioxidant activity and the color (CIELab) parameters were measured. Three sets of extracts were kept for 2 weeks at -2 °C; 4 °C and 25 - 30 °C and afterwards the parameters mentioned above were measured once again. Moreover, the total content of polyphenols was determined by Folin-Ciocalteu method. The results were expressed in mg gallic acid equivalents per liter. The antioxidant activity was determined using the method based on the interaction with the ABTS radical, the results being expressed in mmol trolox equivalent per liter. Thermal treatments have not influenced antioxidant activity, but have significantly affected the extract by increasing its luminosity and changing the red/green parameter towards more red tones, while storage at -2 °C caused the decrease of yellowness. Storage at 4 °C was found to be optimal for the preservation of the antioxidant activity.

Keywords: *ABTS, CIELab, dog-rose extract, food dye, temperature*

INTRODUCTION

Nowadays the consumers worldwide are becoming more aware of the relationship between diet and health. Furthermore there is an increasing fear of synthetic ingredients and it has been several years already since the food industry started to adapt to the demand of consumers and to replace synthetic food dyes with fruit and vegetable concentrates [1]. For this reason the producers of food additives are always looking for natural sources of coloring substances which could provide new color tones which would also be stable [2]. Wild berries present a potential source of such substances that present both a high technological value and functional health value.
Rose hips have been used for centuries in many foods and drinks such as teas, jellies, jams and alcoholic beverages. The plant is also used as a traditional medicine for ailments like colds, flu, inflammations, chronic pain and ulcers [3 – 5]. In French folk medicine, the flower is used to treat scurvy and haemorrhoids, also as an antihelmintic and fortifying agent. The flower is also used in Bulgaria to cure diseases of the gastrointestinal tract. In Russia, it is recommended for the treatment of upper respiratory tract infections. Dried rose fruits can also be used to treat all ailments caused by vitamin C deficiency such as diarrhoea, low activity of the gastrointestinal tract, etc. [5]. The berries of dog-rose (*Rosa canina*) have a high content of polyphenols and carotenoids – compounds with intense coloring and antioxidant properties, thus the extracts from such plants could serve as technological aid in various food processes.
The aim of this study is to research the stability of the antioxidant activity and color parameters during various thermal treatments and storage at different temperatures in order to provide answers for food processors and professionals working in the field of new product development.

MATERIALS AND METHODS

The hips of dog-rose *(Rosa Canina)* were harvested in the Republic of Moldova. ABTS was obtained from Alfa Aesar, Germany and Folin-Ciocalteu reagent was purchased from Merck, Germany. After harvest, the hips were dried at the temperature of up to 65 °C, chopped to a powdery state and sieved. The extraction was performed in 50 % ethanol solution, at the ratio 1g/10mL of solvent. The mixture was stirred for 30 min at room temperature in order to maximize the extraction. The extract was afterwards submitted to the following thermal regimes: -2 °C for 12 hours; 4 °C for 12 hours; 40 °C for 15 minutes, 60 °C for 15 minutes, 80 °C for 15 minutes and 100 °C for 2 minutes, after which the antioxidant activity and the color parameters (CIELab) were assessed. Triplicates of three sets of samples were kept for 2 weeks at -2 °C; 4 °C and 25 - 30 °C and afterwards the parameters mentioned above were determined once again.

Antioxidant activity by reaction with ABTS radical

The antioxidant activity was measured using the reaction with ABTS radical. The ABTS radical was produced from 7 mM ABTS solution, by mixing it with 2.45 mM potassium persulfate. The aforementioned mixture was left in the dark, at room temperature for 12 - 16 hours before use. Before the analysis the radical solution was

diluted to the absorbance of 0.70 (± 0.02) at 734 nm while the samples were diluted so they would produce 20 % - 80 % inhibition of the blank absorbance. 1.0 mL of diluted ABTS radical solution were added to 10 µL of extract or trolox standard, after which the absorbance reading was taken between 1 min after initial mixing and up to 6 min, using ethanol as a blank. The results were expressed as mmol trolox equivalents per liter (mmol TE·L^{-1}) from a curve constructed using trolox (0 - 2000 µM, R^2 = 0.9805) [6].

Total polyphenols by Folin-Ciocalteu

The total content of polyphenols was determined using the reaction with Folin-Ciocalteu reagent. For the analysis 0.2 mL of previously diluted sample were introduced in a test tube, after which 6 mL of distilled water and 0.5 of Folin-Ciocalteu reagent were added. The mixture was vortexed. After 1 min, 1.5 mL of aqueous sodium carbonate (20 %) were added, the mixture was vortexed again and allowed to stay in the dark at room temperature for 120 min. The absorbance was read at 750 nm using an optic glass cuvette of 1 cm path length against a blank prepared with distilled water instead of the sample. The results for total polyphenols were calculated from a calibration curve using gallic acid (0 - 500 mg·L^{-1}, R^2 = 0.9988) and were expressed in equivalents of gallic acid per liter (mg GAE/L) [7].

Color parameters (CIELab)

The CIELab parameters were determined by reading the transmittance spectra of the extracts between 380 nm and 780 nm, every nm, using the Analytic Jena Specord 200 Plus spectrophotometer (Germany) and the optical glass cuvette of 1 cm path length. Distilled water was used as reference. The calculations were made using the Specord programme provided by the same company. The chosen illuminant was D65 with the observer placed at 10°.

Statistical analysis

The mean values and the standard deviations were calculated from 3 parallel experiments. ANOVA and post-hoc Tukey test were used to distinguish between means and evaluate the results. The considered significance level was $p \leq 0.05$. The calculations were performed using IBM SPSS Statistics 23.

RESULTS AND DISCUSSION

The influence of temperature on the antioxidant activity and color parameters of chokeberry extract

The total polyphenol content, the antioxidant activity, and color parameters of the initial extract are presented in Table 1.

Table 1. *Initial polyphenol content, antioxidant activity and color parameters of chokeberry extract (the results are expressed as means ± standard deviations)*

Indices	Values
Total polyphenols, mg GAE·L^{-1}	4776±43
Antioxidant activity, mmol TE·L^{-1}	40.26±0.33
Luminosity (L*)	92.29±0.03
Red/green component (a*)	0.51±0.01
Blue/yellow component (b*)	18.30±0.07
Chroma (C*)	18.31±0.07
Hue (H*)	-0.07±0.11

It was found that the extract contains a high amount of polyphenols i.e. 4776 mg·L^{-1} which most probably contribute to the fairly high antioxidant activity namely 40.26 mg TE·L^{-1} and deep red-yellow color. The main color factor for rose hips are however the carotenoids [8]. The red/green component suggest a predominance of red tones in the color of the extract, while blue/yellow the presence of yellow pigments. It has been reported that rose hips lose their yellow tones during ripening [8] which is why the moment of harvest is important for an optimal extract in terms of its color quality.

Of the six species of *Rosa*, namely *Rosa canina*, (*Rosa dumalis* subsp. *boissieri*, *Rosa dumalis* subsp. *antalyensis*, *Rosa villosa*, *Rosa pulverulenta* and *Rosa pisiformis*), analyzed by Ercisli (2007) [3], *Rosa canina* (rose hip) was the one with the highest total phenolic content (96 mg GAE/g DW). The numbers found by Erclisi [3] are comparable to the result of this study when the latter is recalculated to the mass of dry weight. The same author also concluded that the content in total phenolics may vary depending on the species and the growing conditions. The extraction and sample preparation techniques are some other factors that may affect the final concentration of the extract [8]. Czyzowska et al. (2015) [5] have analyzed wine made from rose hips and found that phenolic levels ranged from 2786 to 3990 mg·L^{-1} while the antioxidant activity analyzed by DPPH radical ranged from 8 to 13.5 mM·L^{-1}.

The results presented in Figure 1 show that neither of the tested thermal treatments has significantly affected total antioxidant activity. It would be however interesting to study if the antioxidant activity is due to polyphenols or is it imparted by the vitamin C normally found in rose hips. Similar results were found during the research of the influence of temperature on grape marc [9] and chokeberry extracts (unpublished data). Several other studies on the effect of temperature and time have been published. Patras et al. (2009) [10] studied the effect of both high pressure treatment and conventional thermal processing on the antioxidant activity, the levels of different classes of antioxidants and color of strawberry and blackberry purees and found that conventional thermal treatment reduces the levels of ascorbic acid, anthocyanins, antioxidant activity and the color quality, particularly the redness. Another study revealed similar findings in carrot and tomato purees. Only the levels of phenolics remained unaffected after thermal treatment [11]. These studies could provide an explanation for the stability of the antioxidant activity in the extracts prepared from dried berries by assuming that its value is due to the more stable phenolics and carotenoids which have not degraded in the drying process.

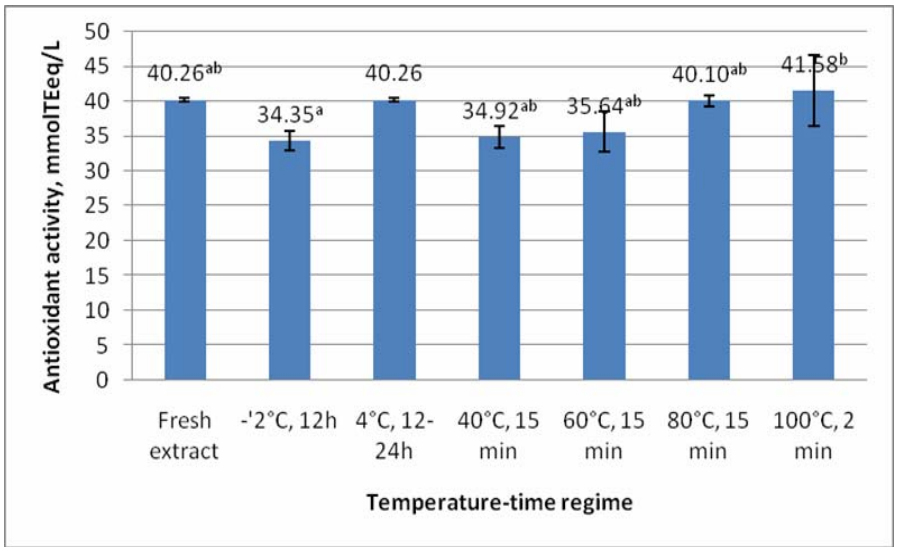

Figure 1. *The influence of temperature on the antioxidant activity of dog-rose ethanolic extract (the results are expressed as means ± standard deviations, different letters designate significantly different results)*

Table 2 shows that temperature below 0 namely -2 °C as well as prolonged thermal treatment even at temperature which are not so high i.e. 40 °C for 15 min; 60 °C for 15 min, have increased the luminosity of the extract and have lightened the extract.

Table 2. *The change of colour parameters during various thermal treatments (the results are expressed as mean ± standard deviation, different letters designate significantly different results)*

Temperature-time regime	L*	a*	b*	H*
Fresh extract	92.29±0.03^{a}	0.51±0.01^{b}	18.30±0.07^{b}	-0.07±0.11^{ab}
-2 °C, 12 h	95.93±0.10^{b}	-0.17±0.08^{a}	14.60±0.06^{a}	11.54±17.45^{b}
4 °C, 12-24 h	92.29±0.05^{a}	0.51±0.01^{b}	18.30±0.07^{b}	0.17±0.08^{ab}
40 °C, 15 min	96.65±0.10^{b}	-0.32±0.09^{a}	14.10±0.07^{a}	1.36±1.40^{ab}
60 °C, 15 min	96.08±0.36^{b}	-0.28±0.06^{a}	15.05±0.24^{a}	-1.48±2.15^{ab}
80 °C, 15 min	90.96±1.82^{a}	0.71±0.32^{c}	18.72±0.86^{b}	0.06±1.20^{ab}
100 °C, 2 min	92.27±1.38^{a}	0.49±0.06^{b}	17.69±0.84^{b}	0.39±1.87^{ab}

The same treatments have also led to the degradation of red pigments and an evolution of color towards greenish tones. Temperatures bellow 0 °C as well exposure for 15 min to temperatures of 40 °C and 60 °C have caused a loss of the yellow quality. All these changes in the aforementioned extracts have produced a decrease of chroma (Figure 2) the parameter which describes the quality of color, its vividness or dullness. Among specialists it is also known as saturation and it indicates how close is the color to gray or to the pure hue [12]. The colors which contain gray pigments are described as less saturated or muted and the numeric values of Chroma are lower than the ones of pure hues.

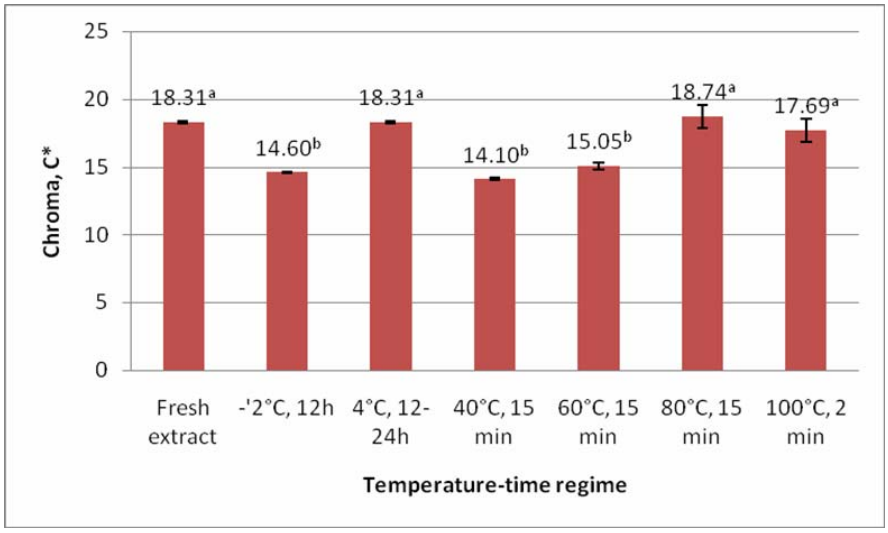

Figure 2. *The influence of thermal treatment on the Chroma of dog-rose extract (the results are expressed as means ± standard deviations, different letters designate significantly different results)*

Figure 3 clearly indicates that the colors of the samples submitted to -2° for 12 hours, to 40 °C for 15 min and 60 °C for 15 min have become duller, although the perceptibility of this phenomenon still has to be evaluated. Interestingly the higher values for Chroma correspond with the higher values of antioxidant activities. However Figure 3 shows that storage at different temperatures does influence antioxidant activity.

The influence of storage conditions on the antioxidant activity and color parameters

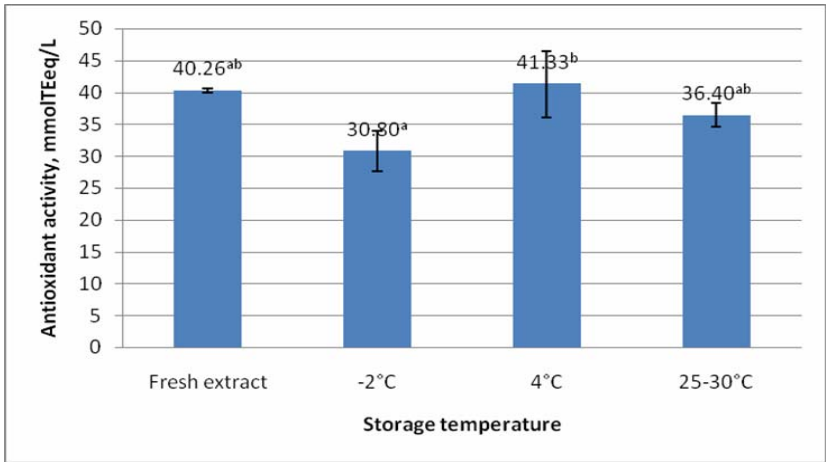

Figure 3. *The influence of temperature on the antioxidant activity of dog-rose ethanolic extract during storage (the results are expressed as means ± standard deviations, different letters designate significantly different results)*

Statistical difference was found between values determined for the extract kept at -2 °C and the one kept at 4 °C. The study has shown that storage at 4 °C is better for the preservation of the antioxidant activity. Furthermore even room temperatures did not affect this parameter as much as the temperature below 0 °C. This stability could be

explained by the stability of different phenolics to the action of temperature. Casati et al. (2012) [13] researched the influence of storage on the polyphenol content and color parameters in processed blueberry, elderberry and blackcurrant juices and found that both the levels of phenolics and the color quality decreased with time. Of course, the longer storage time and the different solvent media should be taken into account when comparing the results of the two studies. Moreover, it would be interesting to research the stability of different extracts prepared from dried berries after their addition into products such as fruit and berry juices.

Table 3 summarizes the results for CIELab parameters after two week storage at different temperature.

Table 3. *The change of color parameters during various storage conditions (the results are expressed as means ± standard deviations, different letters designate significantly different results)*

Temperature-time regime	L*	a*	b*	H*
Fresh extract	92.29 ± 0.03^{a}	0.51 ± 0.01^{b}	18.30 ± 0.07^{bc}	-0.07 ± 0.11^{ab}
-2 °C, 2 weeks	95.78 ± 0.16^{b}	0.06 ± 0.03^{a}	14.42 ± 0.13^{a}	-0.07 ± 0.11^{ab}
4 °C, 2 weeks	90.53 ± 0.43^{a}	0.88 ± 0.08^{cd}	19.08 ± 0.02^{c}	-11.59 ± 7.20^{a}
25 - 30 °C, 2 weeks	94.83 ± 0.05^{b}	1.23 ± 0.01^{d}	19.26 ± 0.01^{c}	0.16 ± 7.79^{ab}

The results have shown that very low temperatures i.e. -2 °C as well as room temperatures i.e. 25 - 30 °C can significantly affect the extract by increasing its luminosity and by shifting its color towards more red tones. Moreover, storage at -2 °C caused the decrease of yellowness. Cunja et al. (2015) [8] have analyzed the CIELab parameters the hips of dog-rose at various stages of maturity and have also observed that frost decreased the redness, the yellowness and increased the luminosity on the other hand. It seems that negative temperatures have a negative effect on the pigments of *Rosa canina* indeed.

The overall quality of color (Figure 4) was not, however, significantly affected. Only storage at -2 °C has decreased its value by almost 4 unities.

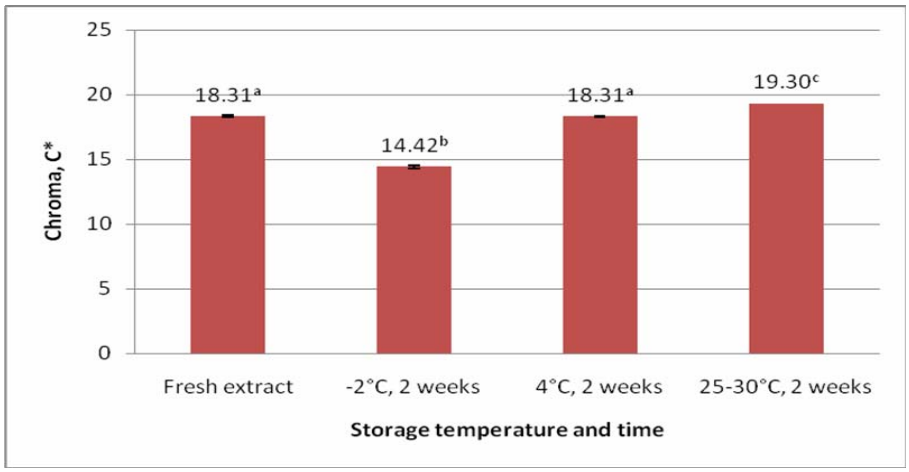

Figure 4. *The influence of storage temperature on the Chroma of chokeberry extract (the results are expressed as means±standard deviations, different letters designate significantly different results)*

Table 4 presents the calculated values for overall colorimetric difference (ΔE*) between the freshly prepared extract and each of the extracts exposed to a certain thermal regime.

Table 4. *Overall color difference between the fresh extract and the extracts submitted to various thermal treatments*

Time-temperature regime	-2°C, 12h	4°C, 12-24h	40°C, 15 min	60°C, 15 min	80°C, 15 min	100°C, 2 min	-2°C, 2 weeks	4°C, 2 weeks	25-30°C, 2 weeks
ΔE*	5.23	0	6.11	5.05	1.40	0.62	5.24	1.96	2.90

The results from Table 4 show that the changes of CIELab parameters of the extracts previously mentioned have resulted in a visible change of the overall color of the extracts. No data on the perceptibility threshold of color change for rose hip extract could be found. Nevertheless, several authors have reported such threshold for wine color i.e. ΔE* = 0.8 - 1 reported by Gonnet (2001) [14] and ΔE* = 3 reported by Martinez et al. (2011) [15]. Some color differences are evaluated differently by the human eye and using ΔE* even though its calculation is based on the simulation of the color vision of the human eye [16]. Thus, in future studies, it would be useful to perform the sensory analysis of the extracts by group of panelists in order to determine the perceptibility threshold and to correlate it with the overall colorimetric difference ΔE*.

CONCLUSIONS

Dog-rose extract contains a significant amount of polyphenols and as a result high antioxidant activity and deep red-yellow color. The experiments on thermal treatments have shown that very low temperatures as well as room temperatures can significantly affect the extract, increase its luminosity and change the red/green parameter towards more red tones, while storage at -2 °C caused the decrease of yellowness.
Storage at 4 °C is optimal for the preservation of the antioxidant activity. Temperatures below 0 as well as room temperatures i.e. 25 - 30 °C can significantly affect the extract by increasing its luminosity and by shifting its color towards more red tones.

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REFERENCES

1. Scott-Thomas, C.: Natural and organic trends drive European food colourings growth. *http://www.foodnavigator.com/*. [Online] October 08, **2014**. [Cited: January 23, 2016.]

http://www.foodnavigator.com/Market-Trends/Natural-and-organic-trends-drive-European-food-colourings-growth;
2. www.confectionerynews.com, [Cited: January 31, 2016.] http://www.confectionerynews.com/Trends/Natural-Colors-Flavors;
3. Ercisli, S.: Chemical composition of fruits in some rose (Rosa spp.) species, *Food Chemistry*, **2007**, **104**, 1379-1384;
4. Lattanzio, F., Greco, E., Carretta, D., Cervellati, R., Govoni, P., Speroni, E.: In vivo anti-inflammatory effect of Rosa canina L. extract, *Journal of Ethnopharmacology*, **2011**, **137**, 880-885;
5. Czyzowska, A., Klewicka, E., Pogorzelski, E., Nowak, A.: Polyphenols, vitamin C and antioxidant activity in wines from Rosa canina L. and Rosa rugosa Thunb, *Journal of Food Composition and Analysis*, **2015**, **39**, 62-68;
6. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C.: Antioxidant activity applying an improved ABTS radical cation decolorization assay, *Free Radical Biology & Medicine*, **1999**, **26**, 1231-1237;
7. Singleton, V. L., Rossi, J A.: Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents, *American Journal of Enology and Viticulture*, **1965**, **16**, 144-158;
8. Cunja, V., Mikulic-Petkovek, M., Zupan, A., Stampar, F., Schmitzer, V.: Frost decreases content of sugars, ascorbic acid and some quercentin glycosides but stimulates selected carotenes in Rosa canina hips, *Journal of Plant Physiology* , **2015**, **178**, 55-63;
9. Cristea, E., Sturza, R., Patra□, A.: The influence of temperature and time on the stability of the antioxidant activity and colour parameters of grape marc ethanolic extract, *The Annals of the University Dunarea de Jos of Galati, Fascicle VI – Food Technology,* **2015**, **39** (2), 96-104;
10. Patras, A., Brunton, N.P., Da Pieve, S., Butler, F.: Impact of high pressure processing on total antioxidant activity , phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées, *Innovative Food Science and Emerging Technologies*, **2009**, **10**, 308-313;
11. Patras, A., Brunton, N.P., Da Pieve, S., Butler, F., Downey, G.: Effect of thermal and high pressure processing on antioxidant activity and instrumental colour of tomato and carrot purrées, *Innovative Food Science and Emerging Technologies*, **2009**, **10**, 16-22;
12. https://www.rgbworld.com/ [Cited: April 04, 2016] https://www.rgbworld.com/color.html;
13. Casati, C.B., Sanchez, V., Baeza, R., Magnani, N., Evelson, P., Zamora, M.C.: Relationships between colour parameters, phenolic content and sensory changes of processed blueberry, elderberry and blackcurrant commercial juices, *International Journal and Food Science & Technology*, **2012**, **47** (8), 1728-1736;
14. Gonnet, J. F.: Colour effect of co-pigmentation of anthocyanin revisited-3. A further description using CIELab differences and assessment of matched colours using the CMC model, *Food Chemistry*, **2001**, **75** , 473-485;
15. Martinez, J. A., Melgosa, M., Perez, M. M., Hita, E., Neguerela, A. I.: Note. Visual and Instrumental Color Evaluation in Red Wines, *Food Science and Technology International*, **2001**, 439-444;
16. http://www.konicaminolta.com/ [Cited: March 14, 2016] http://www.konicaminolta.com/instruments/knowledge/color/part5/01.html.