THESES OF DOCTORAL (PHD) DISSERTATION

APPLICATION OF PHYSICAL METHODS FOR QUANTIFICATION OF COLOURANTS IN CONFECTIONARY PRODUCTS

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The colour is one of the key product attribute of foods besides nutrition, taste, and consistency. The consumers generally prefer the foods in attractive colour; moreover, the quality and quantity of food dyes are strictly regulated. Consequently, great attention must be paid to choose the suitable colorant in the appropriated amount during the processing of foods, particularly in situations where children and young people are primary consumers.

The approved food colorants cover the whole spectral range, and they have a huge variability in terms of origin. These colorants can be natural, nature-identical, or artificial. In Hungary, the application rules are regulated by Regulation (EC) No 1333/2008 of the European Parliament and the Council, defining the product families in which a certain colourant can be applied, and the maximum dosing limits.

Traditionally, HPLC and spectrophotometry are the standard, and most widely used analytical methods for quantification and identification of colorants, which have unrivalled resolution, sensitivity and selectivity. These methods` high installation and running cost, and the complex sample preparation techniques supported the development of further alternative methods for the qualitative and quantitative analysis of food colorants. For example, such methods are the Thin Layer Chromatography (TLC), Capillary Electrophoresis (CE), Colorimetry, or Photoacoustic Spectrometry (PAS).

1. MATERIALS AND METHODS

The main objective of our studies was to investigate the ability of natural and artificial colorant content quantitative determination in confectionary products by mean of non-destructive optical methods, like colorimetry and laser-based photoacoustic spectrometry (LPAS). For that we described functions between the colourant content(s) in confectionary products and the measurable colour characteristics (e.g. CIELab colour indices measured with a reflective spectrocolorimeter, or a photoacoustic signal measured with LPAS), which functions can be used in quality control of the finished products after the validation of the methods. The obtained results were compared to the outcome of parallel studies conducted by spectrophotometry (SP) as classical, reference method.

The defined colorimetric, and photoacoustic calibration lines were used to investigate the composition of the effervescent tablets during the powder mixing process before pressing. For this purpose, two mixing trials were conducted, in which trials samples were taken from different places of the mixer to determine the efficiency of mixing at a certain time. The length of mixing process is proper, if the further continuation of this operation does not change the distribution of the components and thus the concentration of the colorants.

The tested matrixes were confectionary products: hard-boiled candies and effervescent tablets. These samples were made according to the industrial process using additional components like minerals, flavours or acids. In our studies, there were three naturals (anthocyanin, beta carotene, betanin), and an artificial azo dye (Ponceau 4R) investigated.

Five solid hard candy samples were prepared, to test the anthocyanin content of in solid form, which had 0,000 (blank), 0.481, 0.937, 2.001 and 5.695 mg/g anthocyanin in their formula. The anthocyanin calibration standards were made from anthocyanin, and blank sample dissolved in purified water, and the pH was set up with citric acid.

The hard-boiled candy samples containing anthocyanin and beta carotene samples can be divided into two series. Series A contains varying amounts of anthocyanin grape-extract with no other colour added. The anthocyanin content of these samples was 0.00 (blank), 5.06, 8.14, 10.65, 14.75 and 17.42 mg/g. In series B, two colorants were applied, namely the above-mentioned grape extract and liquid beta carotene colorant. The amount of anthocyanin was fixed (9.50±1.20 mg/g), and the beta carotene contents were as follows: 0.00 (blank sample from beta carotene point of view), 0.16, 0.22, 0.37 and 0.42 mg/g.

Two sets of (powdered) effervescent tablet samples were investigated -a series with betanin, and another coloured with Ponceau 4R. The betanin contents of the first series were as follows: 10, 20, 30, 40, 50 and 60 mg/g, and the other series had 0.05; 0.10; 0.15; 0.20; 0.30; 0.40 and 0.50 mg/g Ponceau 4R content. For the mixing process optimization two series of trial samples were created, one series with 30 mg/g of betanin, and the other with 0.50 mg/g of Ponceau 4R. The sampling from the mixture containing betanin happened after 1, 3, 6, 10, 15, 20 and 25 minutes of mixing, and from the powder made with Ponceau 4R, the samples were taken after 1, 3, 6, 10, 15, 18, 20 and 25 minutes.

At first step of the sample preparation for spectrophotometric tests, we added 20 ml sample solution (or calibration standard) into a dark 25 ml volumetric flask, then 5 ml organic solvent, like carbon tetrachloride (CTC) or dichloromethane (DCM), or hexane was pipetted to them depending on the extraction type (**Table 1.**). The extraction of the mixture of calibration standards and the organic solvent was started with a 60 seconds manual shaking and then the samples were centrifuged (3K12, Sigma) for 4 or 15 minutes at 2000 rpm. The spectrophotometric measurements were carried out on the samples taken from the water layer of the solvent. The absorbance was recorded on the 380-700 nm spectral range.

Table 1: Different sample preparation (extraction) ways used for spectrophotometric analysis

Extraction type	Organic solvent	Extraction (manual shaking, s)	centrifuging (min; rpm)
„A”	DCM	60	4; 2000
„B”	Hexane	60	4; 2000
„C”	CTC	60	4; 2000
„D”	CTC	60	15; 2000

The limit of detection (LOD) for colour agent was calculated by dividing the threefold standard deviation of blank or the lowest concentration standard, with the slope of the calibration curves.

A Merck Spectroquant Pharo 100 UV-VIS spectrophotometer was used to record the spectra.

A HunterLab MiniScan XE Plus portable reflex spectrocolorimeter was used to measure CIELab colorimetric indices.

A custom built LPAS system used in this study comprised a modulator, a photoacoustic cell and either a 473 nm diode laser (Changchun New Industries Optoelectronics, MBL-III-473-50, 50mW) or a 532 nm diode laser (Roitner Laser Technik GmbH, GLP-III-532-30, 30 mW). The laser beam was mechanically modulated by a chopper. The velocity of the chopper wheel regulated by the chopper controller (HMS Light Beam Chopper 220) was 17 or 23 Hz. The mechanically chopped beam entered the PA cell through a quartz window 12.7 mm in diameter. The sample chamber was connected though a 3 mm long and 300 μm inner diametric cylindrical tube to microphone chamber, where a miniature (4.2mm×4.75mm) electret microphone (Sennheiser KE 4-211-2) was located. The signal of the microphone (LPAS signal) was processed by a dual phase lock-in amplifier (Stanford SR530).

2. RESULTS AND DISCUSSION

2.1 Results of the investigations of samples containing anthocyanin

We observed, if the candy samples containing colourants are dissolved in water, there was a colloid obtained, due to the oil or flavour compound, and the colloids could not be measured directly by a spectrophotometer. Therefore, the before the spectrophotometric test, the oils (flavours) had to extracted from the calibration standards or sample solutions by organic solvent. Three different solvents (DCM, hexane, CTC) were compared, we defined with CTC can be obtained the best recovery of colorant content of a candy samples, has 2.1 mg/g anthocyanin content in the formula. Out of the three chemical agents, CTC had the highest (93.2%) recovery for standard addition. By increasing the length of the centrifuging, the recovery of the standard addition achieved the 96.5% (**Table 2.**), therefore the further samples preparation was done with the application of the „D" type of extraction.

Table 2.: Spectrophotometric results of the samples containing 2.1 mg/g anthocyanin extracted by different way of extraction (λ=520nm)

Extraction type	normal method (E163 mg/g)	St. addition method (E163 mg/g)	Recovery of St. addition	LOD (mg/g)
„C"	1.630±0.045	1.821±0.051	**89.5%**	0.006
„D"	2.184±0.143	2.263±0.148	**96.5%**	0.028

The spectrophotometric anthocyanin calibration lines were created based on the RABINO-MANCINELLI method. Since all these samples are in liquid form, and the pH value of the solution has a significant

influence on absorbance of the anthocyanin, the pH of the calibration solution and the sample solution was needed to be kept the same.

According to the colorimetric test of calibration standards, and samples, the basic CIELab indices along with the colorant concentration follow a saturation (sigmoid) curve, however in a shorter range these indices are in linear correlation with the colourant content.

To covert the saturation curves of anthocyanin to linear, we created the common logarithm of the basic CIELab indices (lgL*, lga*, lgb*). The usage of a logarithm function is prevented by a negative a* (greenish colour) or b* (bluish colour), since the logarithm of negative values cannot be interpreted on a real set of numbers. This was eliminated by adding a hundred to a* and b*, and thus the logarithm of this obtained values would already be a real number, except for the cases a*=-100, and b*=-100. As the colorant content increases, the brightness index (L*) decreases (the colour of the sample becomes darker), therefore a 2-lgL* transformation was performed to avoid the decreasing function.

Table 3. shows the determination coefficients between colour indices and anthocyanin content for calibration standards on two pH`s (column 2 and 3).

The 2-lg(L*) parameter had the best linear correlation for liquid (dissolved) samples on both pH, and the worst one was Hue.

Table 3.: Comparison the results on the basis of the determination coefficients (R^2) between colour indices and anthocyanin content for calibration standard. Column 2 and 3 (E 163 content: 0.149-2.376 mg/ml; n=3), Column 4 and 5 (E 163 content: 0.135-1.078 mg/ml; n=3) for liquid calibration standards (dissolved samples), Column 6 and 7 for crushed solid samples (E 163 content: 0.000-5.695 mg/g; n=7)

Colour index	Liquid form (pH 2.8±0,1)		Liquid form (pH 2.33±0.09)		Powdered form	
	R^2	LOD (mg/ml)	R^2	LOD (mg/ml)	R^2	LOD (mg/g)
L*	**0.9631**	**0.004**	**0.9905**	**0.001**	**0.9570**	**0.747**
a*	0.8849		**0.9781**	**0.007**	0.8119	
b*	**0.9503**	**0.029**	0.6134		0.6019	
ΔE*	0.5866		0.8300		**0.9591**	**0.842**
Hue	0.0296		0.3531		0.5612	
C*	**0.9064**	**0.004**	**0.9807**	**0.005**	0.7983	
2-lg(L*)	**0.9932**	**0.003**	**0.9990**	**0.001**	**0.9672**	**0.650**
lg(a*+100)	0.8659		**0.9671**	**0.008**	0.7990	
lg(b*+100)	**0.9540**	**0.030**	0.6126		0.6102	

The anthocyanin content of four different commercial candy samples was determined using the spectrophotometric, and three colorimetric calibration lines, which had the best regressions (2-lg(L*), L* and b* indices) at pH 2.8±0.1 (**Table 4**). The colourant content determined by the three mentioned colour indices was 14-28% higher compared to the result obtained with the reference method. The closest result to spectrophotometry was given by the 2-Ig (L*).

Table 4.: Anthocyanin content (mg/g) of commercial candies with 2.1 mg/g colorant content measured in dissolved form by spectrophotometry(SP) and colorimetry (using L, b* and 2-lg(L*) indices) (mean±SD, n=3, pH: 2.8±0.1).*

Sample	Anthocyanin concentration (mg/g) in liquid form			
	SP	Colorimetry		
		L*	b*	2-lg(L*)
F7	2.324	2.942±0.029	3.234±0.043	2.626±0.024
F8	2.332	3.186±0.054	2.333±0.031	2.826±0.045
F9	2.554	3.042±0.024	2.669±0.026	2.708±0.020
F10	2.644	3.421±0.025	3.812±0.013	3.025±0.021
average	**2.463±0.161**	**3.148±0.190**	**3.012±0.589**	**2.796±0.159**

Then, the colorant content of the home-made anthocyanin-containing hard candy samples was determined using a spectrophotometric calibration line measured at pH 2.33±0.09 identical to the sample solution. The anthocyanin content of the samples was also determined by the colour indices L*, a*, 2-lg(L*) and lg(a*+100). For the sample with the lowest amount (0.481 mg/g) of anthocyanin content, there were significantly different results obtained compared to spectrophotometry, which can be explained by the fact that it is not possible to determine the colourant content with the CIELab coordinates in such a small concentration level. The colourant content calculated with L* deviated by 1% from the spectrophotometric result of the sample containing 0.937 mg/g anthocyanin, and it deviated 6% calculated with 2-lg(L*). The same deviations of the L*, and 2-lg(L*) for the sample contained 2,001 mg/g anthocyanin were 27 and 21%, and for the sample with an anthocyanin content of 5,695 mg/g the deviations were 5% and 2%. The colourant contents obtained with other colour indices differed even more from the spectrophotometric results. The results of the studies are shown in **Table 5**.

Table 5.: The anthocyanin content (mg/g) of hard candy samples measured in dissolved form by spectrophotometry (SP) and colorimetry using L, a*, and 2-lg(L*) colour indices (mean±SD, pH: 2.33±0.09)*

Sample	Anthocyanin concentration (mg/g) in liquid form				
	SP	Colorimetry			
		L*	a*	2-lg(L*)	lg(a*+100)
G8	**0.481±0.006**	0.715±0.017	0.197±0.006	0.798±0.014	0.096±0.007
G9	**0.937±0.073**	0.950±0.004	0.615±0.005	0.993±0.003	0.568±0.005
G10	**2.001±0.020**	2.532±0.010	2.510±0.002	2.419±0.010	2.569±0.002
G11	**5.695±0.043**	5.436±0.019	4.808±0.006	5.789±0.027	4.736±0.005

The above-mentioned hard-boiled candy samples were crushed in a mortar, and their CIELab indices were determined. Determination coefficients for linear regression between colorimetric indices and anthocyanin content are shown in **Table 3**. The best correlation of powdered (crushed) samples similarly like the case of liquid samples was achieved for parameter 2-lg(L*) index and the worst was for Hue. Then, commercial candy products were also crushed, and their anthocyanin content was determined using the three calibration lines which had the best linear regressions (2-lg(L*), ΔE* and L*). The obtained anthocyanin contents by colorimetry are with 6-11% higher (depending on the colour indices) than the expected colourant content (2.1 mg/g). The anthocyanin content calculated by 2-lg(L*) index was the closest to this value. The obtained results are shown in **Table 6**.

Table 6.: The anthocyanin content (mg/g) of commercially available candies with 2.1 mg/g colorant content measured in powdered (crushed) form measured by photoacoustic spectrometry (LPAS, mean±SD, n=3) and colorimetry (mean±SD, n=7) using L, ΔE*, and 2-lg(L*) colour indices respectively*

Sample	Anthocyanin concentration (mg/g) in powdered form			
	LPAS	Colorimetry		
		L*	ΔE*	2-lg(L*)
G12	2.292±0.136	2.316±0.021	2.281±0.016	2.204±0.020
G13	2.304±0.143	2.185±0.405	2.174±0.410	2.081±0.394
G14	2.212±0.079	2.507±0.280	2.479±0.274	2.394±0.277
average	**2.269±0.213**	**2.336±0.302**	**2.311±0.300**	**2.226±0.295**

There was no clear correlation between the LPAS signal of liquid samples containing anthocyanin or the other tested colorant and the anthocyanin content, presumably due to the light reflection on the sample chamber (wall effect). The photoacoustic signal of liquid sample can be detected by a needle hydrophone instead of a microphone.

The anthocyanin-containing solid candy samples (home-made, and commercial) previously investigated by spectrophotometry were crushed, then the anthocyanin content of the commercial samples were calculated at 532 nm by the photoacoustic calibration line created based on the home-made samples. The crushed commercial candies samples (G12-G14) had 8% higher colourant content determined by the LPAS signal than the expected value (2.1 mg/g), which is very similar to the colorimetric results, moreover, the standard deviation of LPAS results was even smaller. The results obtained are shown in **Table 6**.

2.2 Results of the investigations of samples containing anthocyanin and beta carotene

Table 7. shows the determination coefficients of the linear regression of the different colour indices for beta-carotene-containing calibration solutions. Except from lg(b*+100), all colour indices were linear functions of colorant content and their determination coefficients were greater than R^2=0.9. There was a proportionality between a* and beta-carotene content (R^2=0.9958).

Table 7.: Comparison the results on the basis of the determination coefficients (R^2) between colour indices and beta carotene content for liquid calibration samples (E 160a content: 0.089 mg/g – 0.913 mg/g; n=3)

Colour indices	Liquid form (R^2)	LOD (mg/g)
L*	**0,9798**	**0,206**
a*	**0,9958**	**0,005**
b*	**0,9236**	**0,017**
ΔE*	**0,9596**	**0,052**
Hue	**0,9759**	**0,014**
C*	**0,9275**	**0,016**
2-lg(L*)	**0,9818**	**0,184**
lg(a*+100)	**0,9965**	**0,005**
lg(b*+100)	0,8912	

The determination coefficients of the linear calibration of the CIELab indices for solid candy sample series containing both anthocyanin and beta carotene are shown in **Table 8**. The samples were tested in powdered and solid (uncrushed) form too. The 2-Ig (L*) index was in linear relationship with the anthocyanin content in the used concentration range (uncrushed: R^2=0.9253. powdered: R^2=0.9575). In the case of solid (uncrushed) samples, the R^2 of the other colour indices was smaller, than 0.9. In powder form, the L* and ΔE* have a linear relationship with colourant content. Similarly, like in the case of the other anthocyanin-containing powder samples described in **Table 3**, there was obtained the highest determination coefficients for L *, for ΔE *, and for 2-lg (L *). It should also be noted that, the determination coefficient was always higher in powder form than in solid form for all colour indices.

There is a linear relationship between b* and the beta carotene content (uncrushed: R^2=0.9253. powdered: R^2=0.9575) in the used concentration range, which was also true for the derived lg(b*+100) index. A good linear relationship between Hue index and beta-carotene concentration was also found over the concentration range investigated, with a relatively high determination coefficient (uncrushed: R^2=0.9550. powdered: R^2=0.9763). The determination coefficient of the linear regression of the other colour indices was worse in both powder and solid form than R^2=0.9.

Table 8.: Comparison the results on the basis of the determination coefficients between colour indices and anthocyanin content (Column 2-5; E 162 content: 0.00-17.42 mg/g) or beta carotene colorant content (Column 6-9; E 160a content: 0.00-0.42 mg/g) for calibration standard (n=5)

Colour indices	Anthocyanin content				Beta carotene content			
	uncrushed		powdered		uncrushed		powdered	
	R^2	LOD (mg/g)	R^2	LOD (mg/g)	R^2	LOD (mg/g)	R^2	LOD (mg/g)
L*	0,8829		**0,9427**	**1,934**	0,2242		0,2502	
a*	0,5877		0,8396		0,3117		0,4591	
b*	0,7101		0,8376		**0,9253**	**0,031**	**0,9575**	**0,015**
ΔE*	0,8846		**0,9497**	**2,009**	0,1981		0,0106	
Hue	0,4048		0,3329		**0,9550**	**0,023**	**0,9763**	**0,009**
C*	0,2073		0,8780		0,3901		0,8114	
2-lg(L*)	**0,9426**	**6,847**	**0,9497**	**1,801**	0,2102		0,2502	
lg(a*+100)	0,5847		0,8321		0,3142		0,4594	
lg(b*+100)	0,7169		0,8427		**0,9289**	**0,032**	**0,9610**	**0,015**

There was a linear relationship between the LPAS signal and the anthocyanin content of the candy samples at both wavelengths with good approximation (473 nm: R^2=0.9764; 532 nm: R^2=0.9570). The relationship between beta-carotene content and the photoacoustic signal measured on the samples was linear only at 473 nm (R^2=0.8484), while at 532 nm the photoacoustic signal was independent of colourant content, which can be explained by that, this wavelength is already relatively far from the absorption maximum of beta carotene (453-456 nm).

2.3 Results of the investigations of samples containing betanin

Betanin content was in a linear relationship with L* (R^2=0.9700), a* (R^2=0.9604), C* (R_2=0.9584) and ΔE* (R^2=0.9655) over the concentration range tested. Among the derived colour indices, 2-Ig(L*) and lg(a*+100) were also linearly dependent on the betanin content, moreover, out of the nine colour indices, of the linear regression of 2-lg (L*) had the best R^2 (R^2=0.9803). The determination coefficient of the linear regression of b* (R^2=0.8270) is worse, than the L*`s. The results of the colour measurements on the series of betanin-containing samples in powdered form are shown by **Table 9**.

Table 9.: Comparison the results on the basis of the determination coefficients (R^2) between colour indices and betanin (E 162) content for powdered calibration samples (E 162 content: 10-60 mg/g; n=3)

Colour indices	R^2	LOD (mg/g)
L*	**0.9700**	**13.794**
a*	**0.9604**	**4.436**
b*	0.8270	
ΔE*	**0.9655**	**14.972**
Hue	0.2314	
C*	**0.9584**	**4.453**
2-lg(L*)	**0.9803**	**12.152**
lg(a*+100)	**0.9571**	**4.613**
lg(b*+100)	0.8280	

There was a good linear (R^2=0.9649), and quadratic (R^2=0.9961) relationship between the LPAS signal measured at 532 nm and the betanin content of the samples. However, the relative standard deviations of the results exceeded 10% in several cases (30 mg/g: 12.23%. 40 mg/g: 10.11%. 50 mg/g: 14.94. 60 mg/g: 12.14%).

2.4 Results of the investigations of samples containing Ponceau 4R

We found a good linear function between Ponceau 4R content and L* (R^2=0.9949), ΔE* (R^2=0.9941) or C* (R^2=0.9957). The a* changed proportionally by the concentration of Ponceau 4R (R^2 = 0.9731). Out of there derived colour indices, two had a linear calibration with higher determination coefficient like 0.9 (2-lg(L*) and lg(a*+100)), and even the R^2 of 2-Ig(L*) was the highest out of all nine colour indices (R^2=0.9960). No good linear correlation was found between Ponceau 4R content b* (R^2=0.7825), or lg(b*+100). The linear regression of the determination coefficient of Hue was slightly worse than 0.9 (R^2=0.8951). The results of the colour measurements on powdered samples containing Ponceau 4R are shown by **Table 10**.

Table 10.: Comparison the results on the basis of the determination coefficients (R^2) between colour indices and Ponceau 4R (E 124) content for powdered calibration samples (E 124 content: 0,01-0,50 mg/g; n=3)

Colour indices	R^2	LOD (mg/g)
L*	**0.9949**	**0.021**
a*	**0.9731**	**0.025**
b*	0.7825	
ΔE*	**0.9941**	**0.022**
Hue	0.8951	
C*	**0.9957**	**0.055**
2-lg(L*)	**0.9960**	**0.020**
lg(a*+100)	**0.9837**	**0.026**
lg(b*+100)	0.7827	

A linear relationship was found between the LPAS signal at 532 nm and the Ponceau 4R content of the samples (R^2=0.9788). The quadratic regression has also a good determination coefficient (R^2=0.9941) at this concentration range.

2.5 Results of the investigations of samples made in mixing trials

According to the results shared in chapter 2.3 and 2.4, only L*, a*, ΔE*, C*, 2-lg(L*) and lg(a*+100) gave a good linear relationship (R^2>0.95) with betanin and Ponceau 4R content. Thus, in the investigation of the mixing trial, we focused only on these parameters. Based on the calibration lines, the expected values of mentioned indices at 95% confidence level was determined for the food supplements contained either 30 mg/g of betanin, or 0.05 mg/g of Ponceau 4R, which values are shown in **Table 11**.

Table 11.: The expected values of samples containing 30 mg/g betanin (E 162) or 0.50 mg/g Ponceau 4R (E 124) calculated by different indices on 95% of confidence level (mean±SD)

Indices	*30 mg/g E 162*	*0.50 mg/g E 124*
L*	73.60±7.19	89.18±0.32
a*	10.57±1.95	5.31±0.05
ΔE*	74.38±7.03	89.38±0.30
C*	10.71±1.96	5.91±0.15
2-lg(L*)	0.187±0.042	0.050±0.002
lg(a*+100)	2.060±0.008	2.022±0.000
LPAS (μV)	644.0±125.2	399.3±28.8

Out of L* indices of the samples with 30 mg/g betanin content made in the mixing trial, only two (3 and 15 min) fell within the 73.60±7.19 interval. The L* values did not show any analytical trend. None of the a*, or C*indices were in the expected range. The colorant content of the samples should be 65-70 mg/g based on the a* and C* values. There was not observed any convergence for a* by increasing of the mixing time. The ΔE* values of six samples fell in the 74.38±7.03 interval. The 2-lg(L*) index of each sample was in the range of 0.187±0.042, due to the large standard deviation of the range (±22%). Also, the lg(a*+100) values of each sample fell in the interval of 2.060±0.008. No trend was observed in the change of this colour index

during the mixing time. The betanin content of the samples would exceed 60 mg/g based on a LPAS calibration line. The relative standard deviations of the results exceeded 10% in several points (3, 10, 20, and 25 min) and even 20% in one case (15 min: 21.42%).

The L* of the samples containing 0.50 mg/g of Ponceau 4R followed a saturation curve with increasing mixing time. and only three L* were in the 89.18±0.32 range (15, 18, and 30 min). A quadratic curve (R^2=0.9849) was fit to the data points, in order to define the vertex of saturation curve, i.e. the mixing time, when L* will not decrease further. The minimum of L* was at 30.98 min according to quadratic regression. Only one a* index fell in the interval of 5.31±0.05. Ahead of the mixing time, the a* followed a saturation curve, which had a maximum at 32.24 min according to quadratic regression (R^2=0.9958). Only three ΔE* fell within the 89.38 ± 0.30 interval. The minimum of ΔE* was at 30,08 min defined by quadratic regression (R^2=0.9826). Only one C* values (15 min) were in the range of 5.91±0.15. C* increased by the mixing time, and the maximum of C* was at 31.16 min according to quadratic regression (R^2=0.9950). Only the 2-lg(L*) indices of the samples taken after 15, 18, 20, and 25 minutes fell in the 0.050±0.002 interval. The minimum of the quadratic regression (R^2=0.9852) of 2-lg(L*) was at 30.92 min. The lg(a*+100) colour index of the Ponceau 4R samples had a linear correlation to the colourant content. The lg(a*+100) index of the samples reached the range of 2.022±0.000 at 13 minutes of mixing. The minimum of lg(a*+100) was at 32.13 min according to quadratic regression (R^2=0.9956).

The LPAS signal of each Ponceau 4R-containing trial samples fell within the 399.3±28.8 range, but in several cases the relative standard deviations exceeded 5% (1, 6, 15, and 25 min).

3. THESES

Thesis 1:

We developed a simple, fast and sensitive spectrophotometric method to determine the anthocyanin content of hard candies. Calibration was performed by a matrix fitting method to eliminate interference caused by the high sugar concentration of the sample solution. Lipids, which caused turbidity of the sample solution and, consequently, significant inaccuracies, even at very low concentrations, were removed by liquid–liquid extraction from the calibration and sample solutions. Out of the apolar solvents tested, carbon tetrachloride was found as the most suitable solvent to remove lipids in one step with almost 100% efficiency. Due to the spectral interferences caused by chlorophyll and its degradation products (Rabino-Mancinelli method), the absorbance at 530 nm was corrected by the absorbance at 657 nm ($A= A_{530}-0{,}250*A_{657}$). During the validation of the developed spectrophotometric method, there were determined its linear range (0.149-2.376 mg/ml), its accuracy (96.5%) and the limit of detection (0.028 mg/g).

Thesis 2:

We defined, 2-lg(L*) index had the best linear correlation with anthocyanin content, in solution (dissolved samples), in solid (uncrushed samples), and as well in powdered (crushed samples) forms of hard candy samples (R^2=0.94-0.99) on the practically important colorant concentration range. The determination coefficient in solutions was the best (R^2=0.9990). For anthocyanin-containing hard candies, the most accurate determination of the colourant content is happening by the 2-lg(L*) index in dissolved samples.

Thesis 3:

We confirmed by measurements, the lg(a*+100) index had the best linear correlation to the colourant content in liquid (dissolved) beta carotene-containing candy samples, while in solid and powdered samples, the Hue had the best correlation. In hard candies containing beta carotene, the most recommended way is to determine the colourant content in the liquid (dissolved) form by lg(a*+100) index (R^2=0.9965).

Thesis 4:

We confirmed by measurements, the best methods for the determination of the anthocyanin colorant content in powdered (crushed) hard boiled candies containing both anthocyanin and beta carotene were by photoacoustic spectrophotometry at 532 nm and by colorimetry using total colour difference (ΔE*). In both cases the determination coefficients were good for anthocyanin (R^2=0.9611 and 0.9497), while poor for beta carotene (R^2=0.01).

Thesis 5:

We confirmed it with measurements, that the beta carotene content can be determined most accurately by colorimetry using the Hue index ($R^2_{anthocyanin}$=0.3329; $R^2_{beta\ carotene}$=0.9763) in a sample containing both anthocyanin and beta-carotene.

Thesis 6:

It was confirmed by measurements on the powdered food supplements (effervescent tablets), that the L*, a*, ΔE* and 2-lg(L*) colour indices and a photoacoustic (LPAS) signal measured at 532 nm were in linear correlation (R^2>0.95) to betanin, or Ponceau 4R content of the samples in the tested concentration range.

4. LIST OF PUBLICATIONS

Publication in a peer-reviewed journal in Hungarian:

1. M KOVÁCS, O DÓKA, R KULCSÁR (2014) Élelmiszer-színezékek színparamétereinek vizsgálata étrendkiegészítőkben színméréssel és fotoakusztikus spektroszkópiával. *Acta Agronomica Ovariensis* 1 43-52

Publications in a peer-reviewed journals in English:

1. M KOVÁCS, O DÓKA, D BICANIC, ZS AJTONY (2017) Application of laser-based photoacoustic spectroscopy and colorimetry for quantification of anthocyanin in hard boiled candy. *Microchemical Journal* 135 100-104
2. M KOVÁCS, O DÓKA, D RICHFIELD (2019) Determination of two color agents in hard boiled candy by laser-based photoacoustic spectroscopy and colorimetry. *Carpathian Journal of Food Science and Technology* 11 126-132

Participation on conference:

1. M KOVÁCS, R KULCSÁR, O DÓKA (2012): Étrendkiegészítők gyártási folyamatának optimalizálása színméréssel XXXIV. Óvári Tudományos Nap (Scientific Day in Óvár). 5 October 2012, University of West Hungary, Faculty of Agricultural and Food Sciences, Mosonmagyaróvár pp. 405-409