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Application of laser-based photoacoustic spectroscopy and colorimetry for quantification of anthocyanin in hard boiled candy

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ABSTRACT

The analytical performance of the newly proposed laser-based photoacoustic spectroscopy (LPAS) and colorimetric method for quantification of anthocyanin (E163) in commercially available hard boiled candies are compared to that of the spectrophotometry (SP). Both LPAS and colorimetry are direct methods that unlike SP do not require the extraction of the analyte or some additional sample treatment. Results indicate that LPAS and colorimetry are both suitable for quickly screening content of anthocyanin in hard boiled candies. The correlation between the two methods and spectrophotometry is linear with $R^2 = 0.9989$ for LPAS and $R^2 = 0.9570$ for colorimetry.

1. Introduction

Because color is, in addition to nutrition, taste, and consistency one of the most important qualitative parameters properties of foods, the quality and quantity of food dyes must be controlled, especially when targeted group of consumers product are concerned children and young people. To meet consumers' needs and to correspond with current regulations [2] the colorants have to cover the whole spectral range. In general, approved food colorants are natural, nature-identical, or artificial. The most important groups of natural colorants include anthocyanin, carotenes, chlorophylls, and betanin [3].

Most properties of the artificial dyes (their higher coloration, heat resistance, chemical stability, consistent quality and supply) are more favorable than the natural ones. However, their unclear physiological and nutritional role prevents their application [1,4].

Carcinogenicity effects of several banned azo dyes in the food industry were already reported in clinical studies [5]. Some authorized azo dyes e.g. tartrazine (E 102), Quinoline Yellow WS (E 104), Sunset Yellow FCF (E 110), azorubine (E 122), ponceau 4R (E 124) and allurared AC (E 129) in presence of benzoates are responsible for hyperactivity in childhood [6] although it was not confirmed by the "Southampton study" in 2007 [7]. Because of the potential health risk of artificial dyes and the consumer's expectation many food producers substitute the artificial colorants with natural ones [8].

The largest group water-soluble dyes of the plants and in particular the angiosperm plants are anthocyanins. More than 60 different types of anthocyanins are discovered so far [9]. Depending on structure and circumstances [10,11] the color of anthocyanins is pink, red, violet, blue or cyan. The most important sources of anthocyanin are grapes (*Vitis* sp.), elderberry (*Sambucus nigra*), red cabbage (*Brassica oleracea*), rosella (*Hibiscus sabdariffa*), grapefruit (*Citrus sinensis*), black chokeberry (*Aronia melanocarpa*) and sweet potato (*Ipomoea batatas*) [12].

The stability of anthocyanin depends on chemical structure, concentration, pH, temperature, light and the presence of oxygen, enzymes, flavonoids, proteins, and metal ions [3]. Stability increases significantly by encapsulation for example with maltodextrin [13].

There are several analytical methods for quantification of anthocyanins: examples are column and thin-layer chromatography [14,15], HPLC [16–20], spectrophotometry [21,22], capillary electrophoresis [23], diffuse reflectance spectroscopy [24]and colorimetry [16,22].

Since all of these require samples to be presented in liquid form, the pH value of the solution has a significant influence on results [20,25]. Likewise, these methods require sample preparation, use of chemicals and also are time-consuming. The objective of the study described in this paper was to explore the feasibility of laser-based photoacoustic spectroscopy (LPAS) for a direct quantification of E163 in commercially available hard boiled candy samples. The results obtained were compared to the outcome of parallel studies conducted by established

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classical methods such as spectrophotometry (SP) and colorimetry. The measurements were carried out in solids as well as in dissolved samples.

2. Materials and methods

2.1. Standard solutions, candies

Initially, a series of concentration standards needed for calibration of SP and LPAS measurements was prepared. The anthocyanin calibration standards were made from grape-extract (Chr. Hansen; AC 12 WSP) dissolved in distilled water. Overall, six external calibration standard solutions were prepared with (concentration of E163) ranging from 0.149, 0.297, 0.594, 1.188, 1.782 and 2.376 mg/mL. The pH value of every solution was 2.8 ± 0.1.

The sample investigated in this study is a hard boiled candy commercially available from a Hungarian producer. It is known that candies contain six different constituents (according to the label) among which E163 is the only colorant; its concentration is 2.1 mg/g.

The solid calibration standards were prepared according to the industrial process using glucose syrup, granulated sugar, and water. The mixed sugar base solution was evaporated (cooking temperature 145 °C, vacuum pressure: 0.213 bar). Then citric acid, flavor and varying amount of anthocyanin were added to a hot but still fluid sugar base and the whole mass homogenized and solidified by cold air (temperature 16 °C). The anthocyanin content in standards was 0,000 (blank sample), 0.481, 0.937, 2.001 and 5.695 mg/g respectively.

2.2. Spectrophotometry

A UV-VIS spectrophotometer (SpectroQuantPharo 100, Merck) was used to record the spectra between 380 and 700 nm with 4 nm spectral resolution. First, the oils (flavors) were removed from the calibration standards and from the sample solutions (22.91–23.11 g candy was dissolved in 100 mL distilled water) by extraction with carbon-tetrachloride (Merck, pro anal). The extraction started with a 60 seconds manual shaking. Then the samples were centrifuged (3K12, Sigma) for 15 min at 720 rcf. The spectrophotometric measurements were carried out on the samples taken from the upper (water) layer of the solvent.

The Rabino and Mancinelli method [26] was used to determine the absorbance of the samples. According to this method the final absorbance of the samples is given by the following:

$$A = A_{530} - 0.250A_{657}$$

where A_{530} and A_{657} are the absorbances at 530 nm and 657 nm respectively.

2.3. Colorimetry

A MiniScan XE Plus (HunterLab) colorimeter was used to measure colorimetric indices. The light source was a CIE D65 xenon lamp and measuring geometry was the standard 45/0°.

The results of the colorimetric measurements were expressed in the CIELab color space. The L* indices represent the lightness of the sample on the 0–100 scale where 0 is the black, and the 100 is the white. The positive a* represents the red content and the negative a* represents the green content of sample on the red/green axis. The positive b* represents the yellow content and the negative b* represents the blue content of the sample on the yellow/blue axis.

Chroma (C*), is concerned with the quantitative attribute of colorfulness and used to determine the degree of difference of a hue in comparison to a gray color with the same lightness. The higher the chroma value, the higher is the color intensity of samples perceived by humans.

Hue index ($h°_{ab}$) means a color vector's degree of rotation from the positive a* axis in the CIELab color system. The total color-difference (ΔE*) represents the distance between two colors in the color space.

2.4. Photoacoustic spectroscopy

In the photoacoustic spectroscopy (PAS) the sample to be investigated is irradiated by the modulated beam of radiation. The fraction of the energy absorbed by the sample is converted to heat as a result of which the temperature of sample oscillates periodically at a frequency identical to that of the modulated radiation itself. Generated thermal waves eventually reach sample's surface and cause a periodic heating and cooling of the contacting layer of the surrounding gas. Finally, the expansions and contractions of the gas give rise to acoustic waves; these are detected as a voltage (termed photoacoustic (PA) signal) by means of the microphone. The optical and thermal parameters of the sample and the contacting gas all play a decisive role in the generation of PA signal. In order to eliminate the variation of the output power on the emission wavelength of the illuminating source, it is customary to normalize the PA signal from the sample to that obtained (under identical experimental conditions) from the carbon black powder acting as a strongly absorbing reference sample. Being a ratio, such normalized signal is expressed in arbitrary units (a. u.). The homemade PA spectrometer used in this study comprised the 1000 W Xe lamp (Oriel Technology), monochromator (Jobin-Yvon, H-10, spectral resolution of 16 nm), a modulator and a homemade PA cell (Fig. 1a). After passing through the monochromator, the collimated beam of mechanically chopped (17 Hz) radiation was collected by a quartz lens and focused into the PA cell. Radiation enters the PA cell through a quartz window 12.7 mm in diameter. A 3 mm long capillary (inner diameter 300 μm) connects the miniature electret microphone (Sennheiser KE 4-211-2) with the part of the cell that accommodates the sample. The sensitivity of the microphone is 10 mV/Pa at 1000 Hz. The PA signal was processed by a dual phase lock-in amplifier (Stanford SR530) with 3 s time constant coupled to the computer.

Another PA set-up (Fig. 1b) that was used in our study makes use of a diode laser Roithner GLP-III-532-30. The c.w. power emitted by this laser at 532 nm (fixed, monochromatic radiation) is substantially higher than that expected from the continuously tunable Xe lamp used as a source in the monochromator mentioned above. Because of a direct proportionality between the magnitude of the PA signal and the incident power of the excitation source, the availability of a strong radiation source will assist in reaching a better signal.

In all methods, three independent analyses were performed and the final results expressed of the averages of the independent measurements.

The limit of detection (LOD) for the color agent was calculated by dividing the threefold standard deviation obtained with the present experimental set-up and selected analytical wavelength from a blank (i.e. without the color agent in the sample), with the slope of the calibration curves.

3. Results

As the first step, the absorbance of calibrating standard solutions was recorded in the 380–700 nm spectral range. The maximum of absorbance was observed at 525 nm. The calibration line was created and plotted in Fig. 2 based on the Rabino and Mancinelli method.

Next, the spectra of four commercially available candies were recorded and their anthocyanin content determined. The obtained results are shown in the first column of Table 1.

As a next step one has determined the CIELab indices of the calibrating standard solutions and of the commercially available candies. Directly L*, a* and b* indices were measured while colorimetric indices (C*, hue, ΔE_{ab}) calculated or new indices created (2-log(L*), log(a* + 100)). The second column of Table 2 shows the determination coefficients of linear regression for content. The 2-log(L*) index shows the best correlation while hue is the worst.