

Exploiting direct and indirect methods for the estimation of the total carotenoid concentration in dried pastas

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Abstract The total carotenoid concentration (TCC) of several commercially available dried pastas prepared with or without eggs was assessed by means of the two well-established *destructive* approaches [spectrophotometry (SP) and high-performance liquid chromatography (HPLC)] and three *non-destructive, direct* (i.e., no sample preparation required) methods (resonance Raman spectroscopy, photoacoustic (PA) spectroscopy and colorimetry). The results obtained by the three direct methods correlate well with the TCC of investigated dried pastas assessed by SP and HPLC. The best linear correlation and detection limit were achieved for the PA method at 470 nm.

Keywords Pastas · Photoacoustic spectroscopy · Resonance Raman · Colorimetry · Total carotenoid concentration

Introduction

The factory-made pasta available in many different sizes and shapes [1] is completely dried before packaging. Most frequently it is prepared from semolina flour, water and salt, but there are also pastas produced from flour and seasonings. Being firm and containing eggs, dried pasta can be stored over long periods without refrigeration or freezing.

The color, an important quality aspect of pasta [2], depends mainly on the intrinsic content of the flour's natural yellow pigment [3] as well as on the number of eggs and the amount of carotenoids in egg yolk [4, 5]. In general, dried pasta prepared without the eggs has a less pronounced yellow color than pasta containing eggs. Furthermore, the carotenoid profile of wheat flour depends on the specific variety (botanical origin, distribution in kernel, the content of *Triticum durum* Desf, growing conditions) and on processing conditions [6]. For a long time the content of durum's wheat "yellow pigment" is regarded as indicative for color quality. The amber-yellow color of semolina flour is due to natural carotenes and xanthophylls (in particular lutein) in wheat. Increased concentration of these pigments leads to a brighter yellow color; however, semolina flour rich in pigment does not necessarily lead to yellow pasta because the lipoxygenase may affect the extent of yellowness. In addition, the breeding and technological approaches may play an important role in controlling oxidative activity during pasta production [7]. Hence, it is important to have durum wheat that contains low amount of enzymes capable of promoting the

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degradation. On the other hand, the feed [8] and few other parameters [9], influence carotenoid profile in eggs. As an example, enhanced color and increased carotenoid content were found in the yolk of eggs from laying hens fed with carotenoids. Dominating carotenoids found in yolk of chicken eggs are lutein and zeaxanthin [10]. Pigments such as capsanthin, lycopene (tomato), astaxanthin, etc. are also found when hens are fed with specific food supplements. If formulated for enhanced availability and absorption by poultry's digestive system, zeaxanthin (dominant in corn) and lutein are deposited more efficiently in eggs than other carotenoids. Pending the nutritional pattern of laying hens, the total concentration of carotenoids (TCC) in egg yolk spans the range from a few to 25 mg/kg [10, 11]. For example, normal eggs and the products derived from eggs contain less than 10 mg/kg lutein which is considered insufficient to induce beneficial health effects. Therefore, fortification of eggs with the carotenoids is one way of securing adequate levels of dietary carotenoids in eggs and egg pastas.

The instrumental methods presently used to quantify yellow pigments in semolina flours, eggs and pastas can be classified into the two major categories. First one includes imperative, tedious and costly extraction of carotenoids before the sample can be analyzed by either spectrophotometry (SP) or high-performance liquid chromatography (HPLC) [12, 13]. To second category belong the methods the application of which requires no sample pretreatment: examples are reflectance spectroscopy and colorimetry [14], resonance Raman spectrometry and a diversity of photothermal (PT) methods [15]. By virtue of their operational principles these *direct* methods are all potential candidates for the rapid assessment of TCC in pasta.

The main objective of the study described here was to explore the feasibility of direct methods for assessing TCC of pastas produced with and without eggs. The availability of a low-cost, *direct* method as the tool for rapid screening/control of TCC would be greatly appreciated. Thirteen commercially available dried pastas were studied by means of colorimetry, resonance Raman spectrometry and the Xe-lamp-based photoacoustic (PA) spectroscopy as a representative of PT methods. The data obtained was compared to that acquired by SP and HPLC.

Experimental methods

PA spectroscopy

In general, the PA spectroscopy [16–18] implies a selective illumination of the condensed phase sample (enclosed in the chamber called the PA cell) with the periodically modu-

lated radiation. Due to radiationless transitions some part of incident energy absorbed by the sample will be converted into heat. As a result, the sample warms up and cools down at a frequency of modulation. The generated thermal waves reach the sample's surface causing the periodic heating and cooling of the gas layer above the sample. Since volume of the gas in the PA cell is constant, these expansions and contractions give rise to an acoustic wave that is eventually detected by means of the microphone (at the modulation frequency) as the PA signal. The optical and thermal parameters of the sample and of contacting gas play a decisive role in the generation process of the PA signal. To eliminate the effect of the wavelength-dependent changes in the output power of the excitation source, the PA signal measured from a sample is usually normalized to a PA signal obtained under identical conditions from a carbon black powder (strongly absorbing reference). Main advantages of the PA method can be summarized as follows: (1) there is no need for sample pretreatment, (2) the approach is non-destructive and (3) powders and completely opaque specimens difficult to analyze by conventional techniques are readily investigated.

The experimental setup used in this study comprises a 1,000 W Xe lamp (Oriel Technology), the monochromator (Jobin–Yvon H-10, spectral resolution of 16 nm), the mechanical chopper and a home-made PA cell. It is customary for the experimentalists to design their own PA cell to optimize the performance for specific application. The PA spectra were recorded between 400 and 550 nm. After passing through the monochromator, the collimated radiation beam (chopped at 17 Hz) was collected by a quartz lens and focussed into the PA cell (see Fig. 1) manufactured from laboratory quartzware. The lower section of the PA cell, actually the conically shaped stopper, serves as a sample tray. The latter is provided with a central hemispherical cavity (diameter 6 mm) to accommodate a finely milled pasta. The quartz entrance window and the microphone (Knowles EA-1954) are incorporated in the upper section of the PA cell. The 300- μm inner diameter capillary 3 mm long was used to achieve the coupling between the air volume (few mm^3) above the sample and the microphone. The signal detected by the microphone was amplified, detected by a dual-phase lock-in amplifier (Stanford SR530) and finally processed by the computer. At each wavelength 512 readouts of the lock-in were taken and the mean value calculated. The sample was then rejected, the tray cleaned and reloaded with a fresh quantity of the same pasta; overall three independent measurements were made with each pasta. The average of three such measurements was taken as the representative PA signal. Following the normalization to a carbon black powder (strongly absorbing reference) one obtains the normalized PA spectrum.

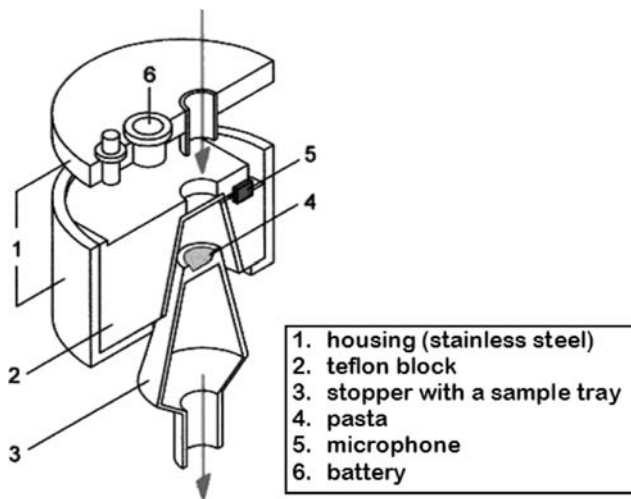


Fig. 1 The exploded view of the home-made PA cell for the non-destructive assessment of TCC in pastas

Resonance Raman spectroscopy

Resonance Raman spectroscopy was already demonstrated a powerful tool for the analysis of carotenoids [19]. Raman scattering is characterized by the three peaks appearing at about 1,523, 1,158 and 1,008 cm^{-1} . These bands originate from the C=C stretch vibrations of the polyene backbone, the C–C stretch vibrations and the rocking motions of $-\text{CH}_3$ groups, respectively [20].

Raman analysis (in the backscattering mode) of pastas was performed with a Renishaw 1000 micro-Raman spectroscope provided with a Leica DMLM imaging microscope and the thermoelectrically cooled CCD array detector. A small quantity of dried, milled pasta was, without any preparation, poured in a tray (diameter 7 mm). The excitation source was the c.w. argon-ion laser emitting approximately 10 mW at 514 nm. The spot (35–40 μm in diameter) of the laser radiation was formed by the 5 \times microscope objective lens (focal length about 13 mm). The Raman spectra were recorded in the range extending from 950 to 1,850 cm^{-1} at spectral resolution of 1–2 cm^{-1} . The 520 cm^{-1} line of the crystalline silicon served to calibrate the frequency scale of the instrument. The intensity of Raman peak at 1,523 cm^{-1} was used as indicative for the concentration of total carotenoids in pasta.

Colorimetry

Color of dried pastas was analyzed with a MiniScan XE Plus colorimeter (Hunter Associates Laboratory, Inc.). This instrument allocates to each sample the color coordinates ($L^*a^*b^*$) within the Commission Internationale l'Eclairage (CIE) Lab three-dimensional color space. In the CIE space L^* represents the brightness ranging from 0 (pure black) to

100 (pure white). The positive/negative values of index a^* refer to the intensity of red/green colorations, respectively. Likewise, the positive/negative b^* values are related to the intensity of yellow/blue coloration. Characteristic for MiniScan XE Plus is the 45°/0° geometry, D65 standard illumination and 10° standard observer. Three to four independent measurements were carried out with each sample and the results expressed as the average of this data.

HPLC

Chemicals and equipment

Analytical grade β -carotene, acetonitrile, methanol and tetrahydrofuran (HPLC-grade) were all obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). The β -carotene was dissolved (0.01 g/50 mL) in the mixture of acetonitrile, methanol and tetrahydrofuran (50:45:5 v/v). The upper solution of beta-carotene diluted at 1 mL/50 mL was used as a working standard in the HPLC measurements. A MILLEX®-HV Syringe Driven Filter Unit (SLHV 013 NL, PVDF Durapore) was purchased from Millipore Co. (Bedford, MA, USA).

Sample preparation

Extracting carotenoids [21] from 2.0 g of finely milled pasta under subdued light conditions was completed within the 2 h using a 2 mL mixture of acetonitrile, methanol and tetrahydrofuran (50:45:5 v/v) and Edmund Bühler SM 30-control shaker (200 rpm/min). The supernatant was decanted and centrifuged in Eppendorf tubes in Hettich Mikro 22R centrifuge (10,000 rpm/min) for 1 min. Finally, the centrifuged extract was filtered on the 0.45 μm Millex-HN Syringe Driven Filter Unit and finally injected onto the HPLC column.

HPLC

Waters high-performance liquid chromatograph purchased from Waters Co. (Milford, MA, USA) is equipped with the 2,487 dual λ absorbance detector, 1,525 binary HPLC pump, column thermostat and 717 plus auto-sampler controlled with EMPOWER™2 software. The installed column was SYMMETRY C18 5 μm 4.6 \times 150 mm; the flow of the eluent (a mixture of ACN, MeOH and THF in proportion 50:45:5 v/v) was 1 mL/min. The pressure on the column was (1,650 \pm 10) psi at 30 °C. The volume injected on the column was 20 μL ; the presence of β -carotene was monitored at 450 nm.

Spectrophotometry

Total carotenoids were extracted by mixing, in a closed 2-mL vial, 500-mg aliquots of milled pasta with 1 mL of

eluent (ACN:MeOH:THF 50:45:5 v/v); this was followed by a 2-min-long vigorous shaking [22]. The suspension was then centrifuged (Sigma 202MC) at 1.0×10^4g for 5 min. Absorbance of supernatant initially measured with the USB-ISS-UV/VIS Spectrophotometer (Ocean Optics, Inc.) was corrected for the background contribution of eluent and the cuvette. The TCC expressed in terms of β -carotene equivalents was calculated from measured absorbance values assuming that all of it is attributed to β -carotene. The relevant specific absorption coefficient ($E 1\% \text{ cm}^{-1}$) for β -carotene in a mixture (ACN:MeOH:THF) at 448 nm was calculated by weighting the individual absorption coefficients in solvents according to $0.5 \times 2,540 + 0.45 \times 2,540 + 0.05 \times 2,399 = 2,533 \text{ dL/g cm}$ [22].

Material

Thirteen commercially available pastas were assigned codes s1–s13. All of them were produced in Hungary and also purchased in Hungarian supermarkets. Besides the pastas prepared without eggs, several egg-fortified pastas have also been studied. Table 1 shows the type of the flour and a number of eggs used to prepare a particular pasta. In fact pasta was produced from what is commonly known as the “egg–liquid”. This latter is obtained by the well-defined sequence of handlings: (1) the egg is cleaned and broken; (2) shell is separated from the yolk and white and (3) finally the yolk and the white are mixed together. According to Hungarian MÉ2-85 regulations, one egg is equivalent to 45 g of such mixture. The numbers 4 and 8 reported in Table 1 (equivalent to 180 g and 360 g egg–liquid mixture) refer to the actual number of eggs added to 1 kg flour. To assure the uniformity of samples when presented for the analysis, the pastas, normally produced in various shapes, were crushed to a fine powder using a coffee grinder; no additional sample preparation steps have been performed.

Since the duration of a drying process and its temperature are important technological parameters, this data whenever available from the manufacturers, is also displayed in Table 1. The range of drying temperatures used with different pastas was wide covering the range extending from 40 to 90 °C. Likewise, for pastas investigated in this study duration of drying process varied between 42 and 16 h. The choice of a drying temperature and the duration of treatment are generally governed by the pasta’s size and shape. For thin and small pasta [such as for example vermicelli, grissini (soup sticks) etc.] lower temperature combined with shorter drying time is used and vice versa.

Table 1 The type of the flour, the number of eggs, the duration of drying treatment and its temperature used to prepare pastas s1–s13

Dried pasta	Type of flour	Number of eggs per kg flour	Drying temperature and duration of treatment
s1	Wheat	Unknown	Unknown
s2	Unknown	8	80 °C for 3.5 h
s3	Wheat	4	90 °C for 4 h
s4	Durum wheat meal	0	Unknown
s5	Wheat	8	40 °C for 16 h
s6	Wheat	8	Unknown
s7	Durum wheat	0	Unknown
s8	Durum wheat	4	90 °C for 4 h
s9	Wheat grist	8	55 °C for 8 h
s10	Wheat	8	Unknown
s11	Wheat	8	Unknown
s12	Wheat grist	8	55 °C for 8 h
s13	Unknown	8	85 °C for 2 h

Results

HPLC

The stock solution containing 0.2 mg β -carotene/mL was used to determine TCC of pastas; the detection wavelength was 450 nm. The retention time of β -carotene is 15.34 min. The area of the peak obtained from a specific pasta was compared to that acquired from the working solution. Likewise, the areas of peaks originating from other carotenoids were calculated and compared to the area obtained from β -carotene standard. In this way one obtains the TCC expressed in terms of β -carotene equivalents. Among the 13 pastas studied here (see Table 1) only 6 (s1, s2, s7, s8, s9 and s12) were found to have TCC detectable by the HPLC. Figure 2 shows the HPLC chromatogram of pasta s12 the TCC of which is 3.38 mg/kg. The β -carotene peak at 15.34 min is preceded by the lutein peak eluting after 14.52 min.

PAS

Figure 3 shows the normalized PA signals between 400 and 550 nm. The normalized PA spectrum peaks at 470 nm; for this reason 470 nm was selected to compare the performance of the PA method to that achieved by other techniques. All 13 pastas have produced PA signals but the spectra of 10 pastas practically overlap; therefore s1 pasta was selected as the representative for this group. The normalized

Fig. 2 The HPLC chromatogram of pasta s12 with TCC of 3.38 mg/kg. The peak eluting at 14.52 min is originating from lutein while the second at 15.34 min is from beta-carotene

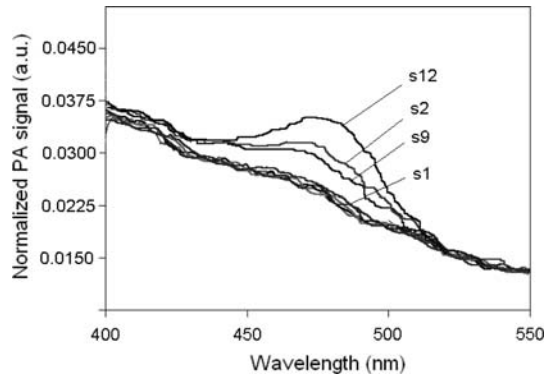
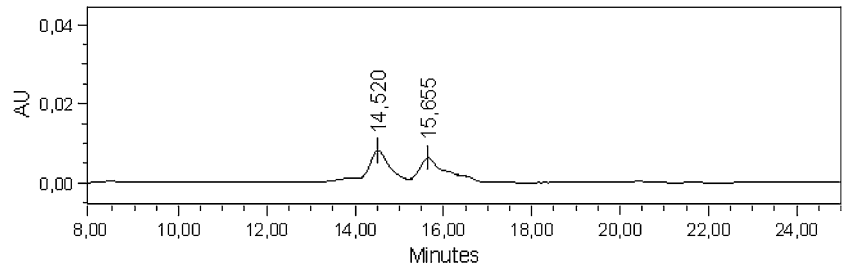


Fig. 3 The normalized PA spectrum of pastas s1–s13 (see also Table 1) in the spectral region between 400 and 550 nm was obtained by rationing the PA signals from these samples to that of the carbon black reference in the same wavelength region. The thus obtained normalized PA signal peaks at 470 nm

spectra of s2, s9 and s12 are clearly distinguished. Measured TCC of pastas s1, s2, s9 and s12 was 0.19, 2.30, 2.45 and 4.04 mg/kg, respectively; the reported values are averages of spectrophotometric and HPLC measurements. The argument for such reasoning is: (1) one and the same mixture of solvents was used for the extraction in both, SP and HPLC measurements, and (2) outcome of SP and HPLC measurements was comparable. The TCC for pastas s7 and s8 was 0.09 and 0.30 mg/kg, respectively. Table 2

contains the correlation coefficient between the PA signal and the TCC; such relationship is highly linear with $R = 0.9966$. The normalized spectra in Fig. 3 indicate dependence of TCC on a wheat variety as well as on a number of egg specific parameters (for example feed, duration of drying and its temperature, etc.).

Resonance Raman spectroscopy

The resonance Raman spectra of six pastas are shown in Fig. 4. Three peaks are observed as expected: the weak one (at $1,008\text{ cm}^{-1}$) is due to a rocking motion of $-\text{CH}_3$ groups, while the strongest peak (at $1,523\text{ cm}^{-1}$) originates from the $\text{C}=\text{C}$ stretching vibrations of polyene backbone. The third peak at $1,158\text{ cm}^{-1}$ is associated with the $\text{C}-\text{C}$ stretching vibrations. Table 3 shows that the resonance Raman technique was capable of detecting TCC in pastas s2, s5, s6, s9, s12 and s13. The relationship between the magnitude of the Raman signal at $1,523\text{ cm}^{-1}$ and the TCC (average of SP and HPLC measurements; see remarks in “PAS” that justify such step) is linear ($R = 0.9972$); this trend is similar to the one observed in the PA measurements (Table 2).

Colorimetry

The dependence of the CIE color index b^* (indicative for the yellowness) was investigated as a function of the TCC

Table 2 Analytical performance data for quantification of total carotenoid concentration (TCC) in pastas

Technique	Linearity best fit ^a							DL estimate (TCC, mg/kg)	Measurement repeatability (RSD, %)
	TCC range (mg/kg)/N/n	R/RSS	Slope		Intercept				
			Mean	RSD (%)	Mean	RSD (%)			
PAS (Xe, 470 nm)	0.1–4.0/6/3	0.9966/1.0622	3.462 $\mu\text{V mg/kg}$	4.1	18.120 μV	1.7	0.1	1.3–4.9	
Raman spectroscopy ($1,523\text{ cm}^{-1}$)	0.2–4.0/4/3	0.9972/2.954 $\times 10^4$	441.716 mg/kg	4.3	–	–	0.7	12.6–29.9	
Colorimetry									
b^*	0.1–4.0/6/3–4	0.9468/2.7873	1.358 mg/kg	17.0	20.461	2.4	1.8	0.9–4.9	
$(b^*)^{2.5}$	0.1–4.0/6/3–4	0.9553/1.691 $\times 10^5$	367.575 mg/kg	15.5	1892.392	6.5	1.7	2.2–12.4	

DL, detection limit = $3.3\text{ SD}_{\text{res}}/\text{slope}$; N, number of concentration points; n, number of independent measurements at each concentration; PAS, photoacoustic spectroscopy; RSD, relative standard deviation; R, correlation coefficient; RSS, residual sum of the squares; SD_{res} , residual standard deviation of the regression line close to DL value (except for Raman spectroscopy); TCC, total carotenoid concentration

^a Each sample was analyzed by HPLC and spectrophotometry (2 independent analyses)

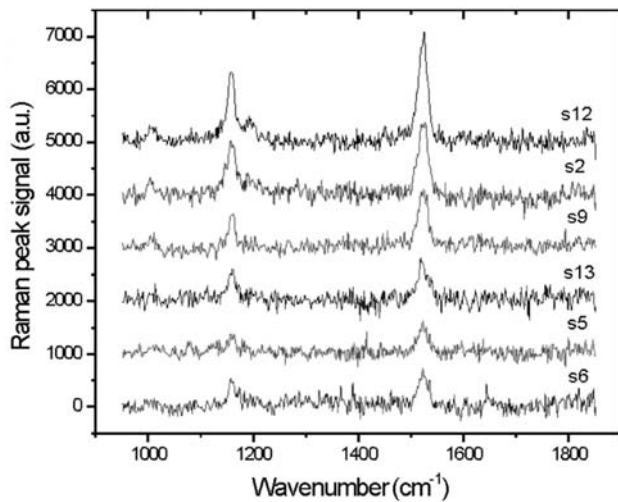


Fig. 4 Resonance Raman spectra of six pastas

Table 3 Raman, PAS and colorimetric data obtained from various pastas

Dried pasta	Intensity of Raman peak at 1,523 cm ⁻¹	PA signal at 470 nm (μV)	Colorimetry <i>b</i> * index
s1	nd ^a	18.61 ± 0.91	19.74 ± 0.96
s2	1,148 ± 224	26.91 ± 0.76	24.64 ± 0.63
s3	nd	18.12 ± 0.68	18.49 ± 0.98
s4	nd	17.46 ± 0.89	19.91 ± 0.17
s5	507 ± 64	18.23 ± 0.65	20.23 ± 0.39
s6	618 ± 125	17.89 ± 0.74	21.47 ± 0.69
s7	nd	18.43 ± 0.69	21.17 ± 0.92
s8	nd	19.20 ± 0.78	20.74 ± 0.95
s9	1,086 ± 226	26.01 ± 0.55	23.85 ± 0.21
s10	nd	18.21 ± 0.73	20.97 ± 0.84
s11	nd	17.76 ± 0.86	17.40 ± 0.30
s12	1,711 ± 512	32.00 ± 0.42	25.35 ± 0.72
s13	680 ± 69	17.62 ± 0.82	19.13 ± 0.37

The values are average of the 3–4 independent measurements ± SD

^a Not detectable

(mean of SP and HPLC results); data in Table 3 shows averages of three to four measurements. The colorimetric index *b** increases at higher TCC and shows a tendency towards saturation. This behavior encountered with the psychophysical-based instrument (as a colorimeter) is not found in PAS and Raman spectrometry where the intensities of the PA signal and of the Raman peak are in a first instance directly proportional to the concentration of the absorbing compound.

The colorimetric data was fitted with the linear, polynomial, logarithmic, power and exponential functions, respec-

tively. As shown in Table 2, the *R* values obtained when fitting *b** data by a linear regression were 0.9468 for *b** and 0.9553 for (*b**)^{2.5}. Due to the fact that SP and HPLC proved capable of detecting carotenoids in the four (s1, s2, s9 and s12) and six pastas (s1, s2, s7, s8, s9 and s12), respectively, only *b** data obtained with these samples have been considered in the analysis.

Discussion and conclusion

The three *direct* methods used in this study all showed a very good performance. The intensity of the PA signal, the value of the colorimetric index *b** and the intensity of Raman peak at 1,523 cm⁻¹ all exhibit highly linear correlations with pasta's TCC (assessed by SP and the HPLC). Surprisingly, not all pastas prepared with eggs produced detectable signals; the satisfactory explanation for this observation has not been found thus far. Most likely, the elevated (above 60 °C) drying temperature and the duration of drying cycle itself are responsible for the degradation of carotenoids in pasta. Furthermore, the process of milling is known to affect the wheat quality; if performed violently such activity may even destroy carotenoid pigment. The incomplete extraction and the fact that the solution might have not been concentrated before the analysis are also potential reasons for lack of HPLC signals.

Raman data shows the presence of carotenoids in s2, s5, s6, s9, s12 and s13 but not in s7 and s8. On the other hand, measurable signals from all 13 pastas were obtained by the PAS method (470 nm and 17 Hz) and colorimetry (index *b**) as shown in Table 3. Highest PA signals and largest *b** values were obtained from pastas the TCC of which was detectable by both, SP and HPLC. This confirms that pastas with TCC < 0.1 mg/kg are not readily detectable either by PAS or by colorimetry (index *b**). In Raman studies highest signals were obtained from pastas richest in TCC (Table 3). In comparison with the resonance Raman spectroscopy, PA is a considerably simpler and less expensive approach because the microphone acts as the universal, wavelength-independent detector eliminating thereby the necessity for the photoelectric devices to detect the signal.

Table 2 displays estimated analytical performances of PAS, Raman spectroscopy and the colorimetric index *b** for quantifying TCC in pastas. Measured PA and Raman signals showed favourable linearity (*R* = 0.997) and a low variability of regression parameters in the range of TCC as high as 4.0 mg/kg. The linear correlation coefficient for parameters *b** and (*b**)^{2.5} was about 0.95. Data presented shows that among the techniques used, PAS emerges as most favourable, both in terms of sensitivity (DL = 0.1 mg/kg TCC) (where DL, detection limit, is the minimum level

at which the analyte can be reliably detected) and repeatability of measurements (RSD 1–5%). The PAS is followed by Raman spectroscopy with DL of 0.7 mg/kg TCC and a much higher imprecision (RSD 13–30%). The precision achieved in the colorimetric studies of index b^* was comparable to that of PA measurements but the estimated DL of 1.8 mg/kg is markedly higher than that achieved by PAS. Moreover, powering of b^* did not improve the sensitivity but reduced precision values by a factor of two (RSD 2–12%). The saturation of b^* and $(b^*)^{2.5}$ values resulted in second order polynomial functions with favourable R value of 0.97. Overall, preliminary data presented in this paper demonstrates that PAS can be proposed as a new analytical tool for a rapid screening/control of the total carotenoid concentration (TCC) in pastas.

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