FTB-3321 *original scientific paper*

Simple and Rapid Quantification of Total Carotenoids in Lyophilized Apricots *(Prunus armeniaca* L.*)* by Means of Reflectance Colorimetry and Photoacoustic Spectroscopy

Ottó Dóka[1*], Gitta Ficzek[2], Svjetlana Luterotti[3], Dane Bicanic[4,5], Ruud Spruijt[4], Ivan Buijnsters[6], György Végvári[2]

[1]Department of Physics and Mathematics, University of West Hungary, Deák F. sq. 1, 9200 Mosonmagyaróvár, Hungary

[2]Department of Fruit Science, Corvinus University H-1118 Budapest, Villányi str. 29-43, Hungary

[3]Faculty of Pharmacy and Biochemistry, University of Zagreb, Ante Kovačića 1, HR-10000 Zagreb, Croatia

[4]Laboratory of Biophysics, Wageningen University, Dreijenlaan 3, 6703 HA Wageningen, The Netherlands

[5]Department of Food Quality and Nutrition, Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottieva 6, HR-10000 Zagreb, Croatia.

[6]Department of. Metallurgy and Materials Engineering, KU Leuven, Kasteelpark Arenberg 44, B-3001 Leuven, Belgium

Received: January 30, 2013
Accepted: July 22, 2013

[*]Corresponding author; Phone: +36 96 566 688; Fax: +36 96 566 620; E-mail: dokao@mtk.nyme.hu

Summary

Photoacoustic spectroscopy (PAS) and reflectance colorimetry are suggested as new analytical tools for analysis of total carotenoids in lyophilized apricot powders. The data obtained by these two techniques from seven apricot cultivars was compared to that acquired by spectrophotometry and high performance liquid chromatography (HPLC).

Best correlations were found between the total carotenoids (TC) content (obtained by VIS spectrophotometry: 1.2-3.4 mg TC/100 g, fresh mass basis) and colorimetric index a* (a* represents the redness of the investigated sample), as well as either Ar-ion laser (or Xe-lamp) based PAS. In all three cases linear correlation was comparable. However, according to the sensitivity and precision data, expressed via limit of detection (*LOD*) and measurement repeatability, the Xe-lamp based PAS is a preferred approach followed by colorimetric index a* and Ar-ion laser PAS.

Both PAS methods exhibit practically the same Pearson correlation coefficient (R=0.987 and R=0.991) values. Nevertheless, residual sum of the squares (*RSS*) and residual standard deviation of the linear regression ($s_{y/x}$) differ markedly. For Xe-lamp based PAS these parameters were much lower than in the case of Ar-ion laser PAS. Likewise, analysis imprecision amounted to RSD of 1-3% for Xe-lamp PAS and 2-6% for Ar-ion laser PAS. On the other hand, as expected, the calibration sensitivity achieved for the PAS signal induced by an Ar-ion laser at 481 nm was substantially higher than that of a Xe-lamp at 470 nm. However, according to much lower $s_{y/x}$ the corresponding *LOD* for Xe-lamp PAS was still two times lower than that of Ar-ion based laser PAS (0.59 *vs.* 1.10 mg TC/100 g). Unlike this, Ar-ion laser PAS showed more favourable instrumental precision and standard error of the weighted mean when compared to Xe-lamp PAS (0.1-0.6 and 0.1-0.3% *vs*. 0.5-8.0 and 0.4-1.7%, resp.). As far as colorimetric indices are being concerned, only a* proved to be analytically useful; excellent R but rather modest *RSS* and $s_{y/x}$ resulting in *LOD* value of 0.70 mg TC/100 g and acceptable analysis imprecision of up to 3%.

The outcome of this research provides sufficient amount of evidence that analytical methods such as reflectance colorimetry and PAS are feasible without the use of any

chemicals for reliable quantification of total carotenoids in freeze-dried apricot homogenates.

*Key words***:** total carotenoids, β-carotene, apricot (*Prunus armeniaca* L.), photoacoustic spectroscopy, colorimetry

Introduction

Different levels of vitamins and antioxidants (carotenoids and polyphenols) in apricots contribute to their taste, colour and nutritive value (*1-3*). Carotenoids and anthocyanins, present in all photosynthetic organisms, are responsible for most of red and yellow colours of fruits and flowers (*4*). In apricot, β-carotene is the most abundant carotenoid exceeding 50% of total carotenoids (TC) content (*5, 6*). Other carotenoids such as α-carotene, zeaxanthin and lutein are also found in apricot fruit but in smaller quantities (*7*).

The content of β-carotene in apricot depends mainly on the variety, ripening stage and the geographical origin. In a recent study performed in Germany on six apricot cultivars (Bergeron-I, Bergeron-II, Harogen, Moniqui, Orangered, and Redsun) the β-carotene varied between 0.1 and 3.9 mg/100 g (*8*). Sass-Kiss et al. (*6*) investigated the carotenoid content of eleven apricot varieties (Royal, Cegledi orias, Gönci Magyar, Sunglo, H-II 25/37, H-II 20/6, Cegledi arany, Cegledi kedvenc, Stella, Mandula kajszi, and Roxana) harvested in Hungary. The quantity of β-carotene found in Royal, Cegledi orias, and Gönci Magyar was 3.80, 3.29 and 3.11 mg/100 g (fresh mass basis), respectively. Likewise, Ali et al. (*9*) studied the TC content of six apricot varieties (Alman, Habi, Khakhas, Mirmalik, Neeli and Shai) grown in Pakistan and TC values ranged between 10.1 (for Shai var.) to 18.1 (for Habi var.), the concentrations are expressed in mg per 100 g of ß-carotene equivalents on dry mass basis.

Significant differences in β-carotene content among Turkish apricot varieties (Hacihaliloglu, Soganci, Hasanbey, Kabaasi, Cataloglu, and Cologlu) were found in a study of Munzuroglu et al. (*10*). The same authors also analyzed wild apricot varieties

and concluded that β-carotene content in these samples was substantially lower than in cultivated products. Based on the color of their flesh, Ruiz et al. (*11*) classified apricots into groups (white, yellow, light orange and orange). Their measurements indicated that a high carotene content of the fruit correlates with the orange color of its flesh, with twice or three times more carotenoids in the skin than in the flesh. It is known that the process of ripening in apricots is accompanied by enhanced biosynthesis of carotenoids (*12*). As an example, Németh et al. (*13*) compared the content of β-carotene in Gönci Magyar kajszi apricot varieties during ripening and observed significant differences between the 60 and 80 % maturity stages.

Petrisor et al. (*14*) established by colorimetric measurements that TC content in apricots increased during the ripening process while chlorophyll a and b decreased drastically. Values of colorimetric indices a* (redness of the sample) and hue angle (h°) [0° means +a* (red) and 90° refers to +b* (yellow) respectively] proved reliable indicators to discriminate between different maturity stages of apricots and to predict their pigment content. In another study changes in the polyphenolic and carotenoid content of three apricot cultivars (Kecskemetska ruza, Madjarska najbolja and Velika rana) grown in two different geographical regions of Croatia have also been investigated (*15*). In all apricot varieties β-carotene content at immature and mature stages differed by one order of magnitude.

Present instrumental methods used to quantify carotenoids in fruits can be classified into two major categories. The first one includes the compulsory, tedious and costly extraction of carotenoids before the sample can be measured by either spectrophotometry (SP) or high performance liquid chromatography (HPLC) (*16, 17*). On the contrary, reflectance spectroscopy, colorimetry, resonance Raman spectrometry and diversity of photothermal (PT) methods, *etc*., are representatives of methods that belong to the second category. They have in common that preparation of the sample is not needed; that is to say the specimens can be studied directly, *i.e*., just as they are (*18-23*). By virtue of their operational principles such direct methods might be potential candidates for a rapid assessment of carotenoids in fruits. On the other hand, the availability of a low cost,

instrument for rapid screening/control of carotenoids would be greatly appreciated in practice.

The main objective of the research undertaken in the work described here was to explore the feasibility of two direct methods for quantification of carotenoids in freeze-dried homogenates of seven apricot cultivars. The methods comprise the colorimetry and the photoacoustic (PA) spectroscopy, both with the Ar-ion laser and Xe-lamp used as excitation sources. The data obtained was compared to that acquired from the same samples by spectrophotometry and HPLC which served as golden standards.

Materials and Methods

Plant growing conditions

The fruits of apricots were taken from the Experimental and Research Farm of the Faculty of Horticultural Science at Corvinus University of Budapest (47º23’ N, 19º08’ E). The orchard at the altitude of 106 m above the sea level is on a light sandy soil with a humus content of 0.8 to 1%. The soil of the Research Field is sandy loam with loess (yellow soil) sedimentation. Based on the 50 year monitoring data, the mean temperature, the number of sunshine hours (per annum) and the average rainfall were 10.8 °C, 2014 h and 500 mm, respectively.

Plant material

The fruits from seven apricot (*Prunus armeniaca* L., syn.: *Armeniaca vulgaris* lam.) cultivars (‘Budapest’, 'Bergeron’, ‘Harogem’, ‘Mandulakajszi’, ‘Pincot’, ‘Sylred’ and ‘Sylvercot’) were examined at 80 % stage of their commercial maturity. The varieties were grafted on myrabolan (*Prunus cerasifera* L. cv. Myrabolana) seedling; trees were planted at a spacing of 2 x 3 m in 2005, the crown form was trained to vase.

Chemicals

The □-carotene standard (CAS number: [7235-40-7]) and all HPLC analytical grade solvents [methanol (MeOH), acetonitrile (ACN), tetrahydrofuran (THF)] were purchased from Sigma Aldrich Chemical Co. (USA). The stock solution was prepared from beta-carotene standard; 0.00229 g beta-carotene was dissolved in 25 mL THF and then diluted

(ten times, final concentration was 0.00916 mg/mL) in a mixture of ACN:MeOH:THF (50:45:5, V/V/V), before injecting on the HPLC column.

Sample preparation

About 10 kg fruits of each cultivar (overall, seven apricot cultivars have been analyzed) were picked (manually) from all four tree quarters of ten trees. Fruits were rinsed with tap water and pitted by hand. Pitted fruits (skins and fruit-pulp) were homogenized using a mixer. Homogenates were first frozen (−25 ºC) and then lyophilized at 1 Pa using ScanVac CoolSafe™ freeze dryer (LaboGene ApS Industrivej, Denmark). Thus prepared lyophilized samples were kept at −25 ºC in darkness until actual analysis.

Sample extraction

The extraction of carotenoids from 5 mg lyophilized apricot powder was carried out under subdued light by adding 1 mL of ACN:MeOH:THF (50:45:5, *V/V/V*) in Eppendorf-tubes and shaking (Edmund Büchler SM 30-control shaker, Germany) at 150 rpm for two hours. The samples in Eppendorf-tubes were then centrifuged (Hettich Mikro 22R centrifuge, Germany) at 10,000 rpm for 5 min at -5 ºC. The supernatant was filtered through 0.45-µm Millex®-HN syringe driven unit filter (SLHV 013 NL, PVDF Durapore, from Millipore Co., USA) and finally injected onto the HPLC-column. This extract was used for spectrophotometric measurements as well.

HPLC analysis

HPLC instrument from WATERS Co. (USA) includes the 2487 dual absorbance detector (analytical wavelength 450 nm), 1525 binary HPLC pump (temperature of the sample compartment set to 5 ºC), column thermostat (set to 30 ºC), in-line degasser AF and 717 plus autosampler (temperature of the sample compartment set to 5 ºC). EMPOWER™ 2 software was used to control the analysis. The column type was SYMMETRY C18, 5 µm, 4.6 x 150 mm. The pressure on the column was 11.55±0.07 MPa and the volume injected into the column was 20 µL. The conditions regarding mobile phase were modified after Bushway (*24*). The mixture ACN:MeOH:THF

(50:45:5, *V*/*V*/*V*) flowing at 1 mL/min was used. The retention time for □-carotene was about 12 min.

Spectrophotometry

The total carotenoid (TC) content was determined from the absorbance measured at 450 nm on a HITACHI U-2800A spectrophotometer (Japan). The samples extracted with ACN:MeOH:THF (50:45:5, *V*/*V*/*V*) and the standard diluted in the same solvent mixture were measured and the TC content expressed on □-carotene basis.

PA spectroscopy

PA spectroscopy (PAS) implies illumination of the condensed phase sample with the periodically modulated radiation of selective wavelength (25). Some part of incident energy absorbed by the sample is converted into heat by means of radiationless transitions. As a result, the sample warms up and cools down at a frequency of modulation. Generated thermal waves reach the sample's surface causing the periodic heating and cooling of the gas layer above the sample. Because the volume of a gas in the PA cell is constant, such expansions and contractions give rise to an acoustic wave. The amplitude of this latter is eventually detected (at the modulation frequency) by the microphone as the PA signal. Optical and thermal parameters of the sample and of contacting gas play a decisive role in the process of PA signal generation. To eliminate the effect of the wavelength dependent variations in the output power of the excitation source, one has to perform a normalization. This is best achieved by dividing the PA signal measured from a sample with a PA signal obtained under identical conditions from a carbon black powder (strongly absorbing reference). Attractive features of the PA method include: i) no need for sample pretreatment and hence shorter analysis time, ii) the approach is essentially non-destructive and iii) completely opaque specimens that are difficult to analyze by conventional techniques are readily investigated by PAS.

An in-extenso description of the PA experimental set-up, used in this study can be found elsewhere (*26*). The PA experiments were conducted using two different excitation sources: Xe-lamp (1000 W, Oriel Technology) with monochromator (Jobin-Yvon H-10, spectral resolution of 16 nm, at 470 nm and 17 Hz) and a c.w. argon-ion laser**,** 481 nm modulated at 30 Hz). Laser power incident on the surface of the sample was 20 mW (*27*).

Apricot lyophilizate powders were investigated by both PA methods directly (*i.e.*, without a need for extraction or other preparation procedure). Measurements of the PA signal were repeated 3-4 times. After rejecting the sample, the PA cell (sample volume 0.25 cm^3) was cleaned (cotton swabs and ethanol) before reloading it again.

Colorimetry

Colour of freeze-dried apricots powder was analysed with a MiniScan XE Plus colorimeter (Hunter Associates Laboratory, Inc, USA). This instrument allocates to each sample specific indices (L*, a* and b*) within the CIE (Commission Internationale l'Eclairage) Lab three-dimensional colour space. In the CIE space index L* represents the brightness that ranges 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, 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. Chroma is the saturation or the vividness of colour. As chromaticity (chroma index, C*) increases, a colour becomes more intense and inversely. Hue angle (h^0) is the basic unit of colour and can be interpreted, for example, as 0° = red and 90° = yellow. Both chroma C* and h° hue are derived from indices a* and b* by means of equations (28):

$$C^* = [(a^*)^2 + (b^*)^2]^{½} \text{ and } h^\circ = \tan^{-1} (b^*/a^*) \text{ (rad)}$$

for metric chroma and metric hue, respectively. Total colour difference (dE*) computed from:

$$dE^* = [(L^*)^2 + (a^*)^2 + (b^*)^2]^{½}$$

is represented by a distance between the absolutely white reference and the sample in the colour space.

Three independent measurements of the same, re-mixed material were carried out with each sample and results expressed as the average of this data.

Data analysis

The goodness-of-linearity fit was documented through Pearson's product-moment correlation coefficient (*R*), residual sum of the squares (*RSS*), and residual standard deviation of the regression line ($s_{y/x}$).

Precision was expressed as instrumental, intrinsic precision of one single measurement based on 512 readouts of the lock-in amplifier, and repeatability from several independent analyses. Standard error of the weighted mean was calculated also.

The limit of detection (*LOD*) was calculated from the residual standard deviation of the regression line ($s_{y/x}$) and the slope of the calibration curve (*S*) according to the formula: $LOD = 3.3 \cdot s_{y/x}/S$ (*29*).

Results

Total carotenoid and □-carotene content in apricots

Table 1 displays the TC content (determined by SP and expressed as □-carotene equivalents in mg/100 g) and □-carotene content (analyzed by HPLC) and the respective standard deviations, found in seven apricot cultivars. Both analytes fit within relatively narrow concentration ranges in available samples (1.2-3.4 mg TC/100 g and 0.6-2.0 mg BC/100 g, fresh mass basis).

The highest content of TC (3.35 mg/100 g) was found in Bergeron cultivar while Sylred and Sylvercot contained the lowest contents of TC (1.22 and 1.23 mg/100 g, respectively). Bergeron is also the richest in β-carotene (2.04 mg/100 g) as compared to a minimum (0.63 mg/100 g) found in Sylred. The last column in Table 1 shows the portion of β-carotene among total carotenoids. With the exception of Mandulakajszi var. (45.7 %) this ratio exceeds 50 % and is highest for Pincot (77.6 %).

Colorimetry

Colorimetric indices L*, a* and b* were measured directly while dE*, C* and h^o have been calculated from this data according to equations stated above.

Linear correlation between the red index a* and TC was higher (R^2=0.9633) than that observed for □-carotene (R^2=0.7493). The former linear correlation is plotted in Fig. 1a. Index b* showed no significant linear correlation *versus* TC with R^2=0.4845; the

same is true for other colorimetric indices with even lower R^2 values. Results are summarized in Table 2.

Data in Table 3 displays the analytical figures of merit. Despite somewhat lower calibration sensitivity (slope of the regression line) for a* *vs*. TC (as compared to b*), the former showed lower imprecision of regression line parameters. Also, higher *R* but lower residual sum of the squares and residual standard deviation of the linear regression values confirm a markedly stronger linear correlation of a* *vs*. TC than b* *vs*. TC and additionaly confirm the lack of linear fit between b* and TC. This was also reflected in almost four times lower *LOD* value (0.70 for a* compared to 2.67 mg TC/100 g for b*). However, nearly comparable repeatability was obtained for both indices a* and b* (RSD ranging 0.4-2.9 %, average values 1.5 and 1.0 %, resp.).

PA spectroscopy

Figure 1b shows the amplitude of the PA signal (Xe-lamp was used as the excitation source) plotted *versus* TC content in apricots lyophilized powders. The relationship between the amplitude of the PA signal and the TC content provides the evidence for linear proportionality (R^2=0.9735) (Tables 2 and 3 and Fig. 1b). The PA signal was in the order of 90 to 120 μV. Each single measurement represents 512 successive readings of the lock-in signal and depends primarily on the power stability of the Xe-lamp. Standard deviation in such "single load" type of measurements ranged from 0.5 to 8.0 %, with an average of 2.2 %, being reflected in standard error of the weighted mean (SEWM) value of 0.4-1.7 %. In the "multi load" type of measurements the achieved relative standard deviation (actually analysis repeatability) that depends on factors such as the stability of the Xe-lamp, the uniformity of samples, *etc*., did not exceed 3 % with an average of 1.9 %.

PA signals obtained in experiments with the Ar-ion laser tuned to 481 nm are shown in Figure 1c. The experimental conditions were similar as in the PA studies conducted with the Xe-lamp. The magnitude of PA signal ranged typically between 3 and 4 mV. The correlation between PA signal and TC content was highly linear (R^2=0.983). Instrumental imprecision based on 512 successive readouts of the lock-in was lower than

1 %, on an average of 0.3 % resulting in a low SEWM value (0.1-0.3 %). Analysis repeatability RSD (with an average value of 4.6 %) did not exceed 6 %.

Output power of the Ar-ion laser at 481 nm is higher than that of the Xe-lamp at 470 nm. This fact might be responsible for higher calibration sensitivity (almost 35 times), higher instrumental precision (on an average ca. 8 times) and lower SEWM values (5-7 times) of the laser based PAS. Obtained *R* values for both PA methods are comparable. Contrary to this, markedly lower *RSS* and $s_{y/x}$ values were achieved for the Xe-lamp based PAS. They undoubtely speak in favour of the higher level of linear correlation *versus* total carotenoids content and resulted in twice lower *LOD* value for Xe-lamp PAS than Ar-ion PAS (0.59 against 1.10 mg TC/100 g).

Discussion and Conclusions

Both, TC and β-carotene content are apricot genotype dependent. Values for the TC content acquired by means of SP and the concentrations of β-carotene determined by HPLC confirm this. As was shown before by Sass-Kiss et al. (*6*), the latter is a dominant carotenoid in apricots. Reflectance colorimetry characterizes the colour of fruit and provides satisfactory estimates of apricot's carotenoid content. Our data for indices a* and b* in freeze-dried apricot homogenates are in accordance with that of Ruiz et al. (*30, 31*) for apricot peel and flesh.

Important to say, statistically significant goodness-of-linear fit was proven for a* and PAS (both with Xe-lamp and with Ar-ion laser) upon *p*-values and *t*-statistic. Correlations between measured colorimetric indices and PA signals against the content of carotenoids are analyte (BC or TC) dependent. As expected, when correlating a* and PA signals with the TC content, the correlations were markedly stronger ($p < 0.01$) than those found when correlating the same parameters to the BC content ($p < 0.05$) (Table 2).

Based on the results emerged, the colorimetry (index a*) as well as both PAS methods (Xe-lamp and Ar-ion laser based) could be used as new analytical tools to reliably quantify TC in lyophilized apricot powders. According to sensitivity and average repeatability data, the Xe-lamp PAS appears to be the most favourable, followed by the colorimetry (index a*) and Ar-ion laser PAS [*LOD* in mg TC/100 g and RSD (%): 0.59 & 1.9, 0.70 & 1.5, 1.10 & 4.6 %, respectively] (Table 3).

All three methods can assist in selecting new varieties of apricots with higher carotenoid content, assessing the extent of their maturity stage and establishing the optimal harvesting time (*14, 22*).

Overall, the outcome of our study suggests realistic prospects for the construction of a compact and affordable instrument applicable for low-cost, reliable and rapid routine analysis of TC in freeze-dried apricots fruit homogenates.

References

1. C.A. Rice-Evans, N.J. Miller, G. Paganga, Antioxidant properties of phenolic compounds, *Trends Plant Sci. 2* (1997) 152–159.
2. J.A. Vinson, Y. Hao, X. Su, L. Zubik, Phenol antioxidant quantity and quality in foods: vegetables, *J Agr Food Chem. 46* (1998) 3630–3634.
3. P.T. Gardner, T.A.C. White, D.B. McPhail, G.G. Duthie, The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices, *Food Chem*. *68* (2000) 471–474.
4. P.M. Bramley: The genetic enhancement of phytochemicals: the case of carotenoids. In: *Phytochemical functional foods*. I. Johnson, G. Williamson (Eds.), Boca Raton, FL: CRC Press (2003) pp. 253-279.
5. M. Radi, M. Mahrouz, A. Jaouad, M. Tacchini, S., Aubert, M. Hugues *et al*., Phenolic composition, browning susceptibility, and carotenoid content of several apricot cultivars at maturity. *HortScience*. *32* (1997) 1087-1091.
6. A. Sass-Kiss, J. Kiss, P. Milotay, M.M. Kerek, M. Toth-Markus, Differences in anthocyanin and carotenoid content of fruits and vegetables. *Food Res Int*. *38* (2005) 1023–1029.
7. P.D. Fraser, P.M. Bramley, The biosynthesis and nutritional uses of carotenoids. *Prog Lipid Res*. *43* (2004) 228–265.
8. C. Kurz, R. Carle, A. Schieber, HPLC-DAD-MS^n characterisation of carotenoids from apricots and pumpkins for the evaluation of fruit product authenticity. *Food Chem*. *110* (2008) 522–530.

9. S. Ali, T. Masud, K. Sarfraz Abbasi, Physico-chemical characteristics of apricot (*Prunus armeniaca* L.) grown in Northern Areas of Pakistan. *Sci Hortic*. *130* (2011) 386–392.
10. O. Munzuroglu, F. Karatas, H. Geckil, The vitamin and selenium contents of apricot fruit of different varieties cultivated in different geographical regions. *Food Chem*. *83* (2003) 205–212.
11. D. Ruiz, J. Egea, M.I. Gil, F.A. Tomás-Barberán, Phytonutrient content in new apricot (*Prunus armeniaca* L.) varieties. *Acta Hortic.* N^{o}. 717 (2006) 363–367.
12. T. Katayama, T.O.M. Nakayama, T.H. Lee, C. O. Chichester, Carotenoid transformations in ripening apricots and peaches. *J Food Sci. 36* (1971) 804-806.
13. S. Németh, L. Szalay, G. Ficzek, M. Stéger-Máté, G. Sándor, Gy. Végvári *et al.*, Analysis of chemical parameters determining the fruit quality of apricot varieties during ripening. *Acta Aliment*. *40* (2011) 109–119.
14. C. Petrisor, G. Lucian Radu, G. Cimpeanu, Quantification of physico-chemical changes during apricot ripening through non-destructive methods. *Revista Chim-Bucharest. 61* (2010) 345-350.
15. V. Dragovic-Uzelac, B. Levaj, V. Mrkic, D. Bursac, M. Boras, The content of polyphenols and carotenoids in three apricot cultivars depending on stage of maturity and geographical region. *Food Chem. 102* (2007) 966–975.
16. H. Müller, Determination of the carotenoid content in selected vegetables and fruit by HPLC and photodiode array detection. *Z Lebens Unters F A 204* (1997) 88-94.
17. E. Biehler, F. Mayer, L. Hoffmann, E. Krause, T. Bohn, Comparison of 3 spectrophotometric methods for carotenoid determination in frequently consumed fruits and vegetables. *J Food Sci*. *75* (2010) C55-C61.
18. M.N. Merzlyak, A.E. Solovchenko, A.A. Gitelson, Reflectance spectral features and non-destructive estimation of chlorophyll, carotenoid and anthocyanin content in apple fruit. *Postharvest Biol Tec. 27* (2003) 197-211.
19. J.K. Hyman, J. Gaus, M.R. Foolad, A rapid and accurate method for estimating tomato lycopene content by measuring chromaticity values of fruit purée. *J Am Soc Hortic Sci*. *129* (2004) 717-723.

20. D. Bicanic, J. Swarts, S. Luterotti, G. Pietraperzia, O. Dóka, H. de Rooij, Direct quantification of lycopene in products derived from thermally processed tomatoes: optothermal window as a selective, sensitive and accurate analytical method without the need for preparatory steps. *Anal Chem. 76* (2004) 5203-5207.
21. M.W. Davey, W. Saeys, E. Hof, H. Ramon, R.L. Swennen, J. Keulemans, Application of visible and near-infrared reflectance spectroscopy (Vis/NIRS) to determine carotenoid contents in banana (*Musa* spp.) fruit pulp. *J Agr Food Chem. 57* (2009) 1742–1751.
22. R.A. Itle, E.A. Kabelka, Correlation between L*a*b* color space values and carotenoid content in pumpkins and squash (*Cucurbita* spp.). *HortScience. 44* (2009) 633–637.
23. D.D. Bicanic, On the photoacoustic, photothermal and colorimetric quantification of carotenoids and other phytonutrients in some foods: a review. *J Mol Struct. 993* (2011) 9-14.
24. Bushway, R. J., 1986. Determination of α- and β-carotene in some raw fruits and vegetables by high performance liquid chromatography. *J Agr Food Chem*. 34, 409-412.
25. A. Rosencwaig: *Photoacoustics and Photoacoustic Spectroscopy*. John Wiley & Sons, New York/Chichester/Brisbane/Toronto (1980).
26. O. Dóka, D. Bicanic, J.G. Buijnsters, R. Spruyt, S. Luterotti, Gy. Végvári, Exploiting direct and indirect methods for the detection of the total carotenoid content in dried pastas. *Eur Food Res Technol. 230* (2010) 813–819.
27. D. Bicanic, D. Dimitrovski, S. Luterotti, K. Marković, C. van Twisk, J.G. Buijnsters *et al.*, Correlation of trans-lycopene measurements by the HPLC method with the optothermal and photoacoustic signals and the colour readings of fresh tomato homogenates. *Food Biophys. 5* (2010) 24-33.
28. G. Wyszecki, W. S. Stiles: *Color Science: Concepts and Methods, Quantitative Data and Formulae*. 2nd Edition. John Wiley and Sons, New York (1982).
29. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, *ICH Harmonized Tripartite*

Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1), Current Step 4 version, ICH, Geneva (November 2005).

30. D. Ruiz, J. Egea, F.A. Tomas-Barberan, M.I. Gil, Carotenoids from new apricot (*Prunus armeniaca* L.) varieties and their relationship with flesh and skin color. *J Agr Food Chem*. *53* (2005) 6368-6374.
31. D. Ruiz, M. Reich, S. Bureau, C.M.G.C. Renard, J-M. Audergon, Application of reflectance colorimeter measurements and infrared spectroscopy methods to rapid and nondestructive evaluation of carotenoids content in apricot (*Prunus armeniaca* L.). *J Agr Food Chem*. *56* (2008) 4916-4922.

Table 1. Total carotenoids and □-carotene content in apricots

Apricot variety	TC by SP (mg/100 g)[a,b]	β-carotene by HPLC (mg/100 g)[a,c]	β-carotene/TC (%)
Sylvercot	1.23 ± 0.02	0.74 □ □0.02	60.3
Pincot	2.00 ± 0.08	1.55 □ □0.02	77.6
Budapest	2.27 ± 0.02	1.24 □ □0.08	54.7
Harogem	2.04 ± 0.09	1.44 □ □0.06	70.7
Mandulakajszi	2.90 ± 0.02	1.33 □ □0.11	45.7
Sylred	1.22 ± 0.02	0.63 □ □0.00$_1$	51.8
Bergeron	3.35 ± 0.14	2.04 □ □0.05	60.9

BC - □-carotene, assayed by HPLC; TC - total carotenoids, assayed by spectrophotometry

[a] Fresh mass basis.

Mean ± SD: [b]*n*=3, [c]*n*=2.

Table 2. Pearson's correlation coefficient (*R*) for linear fitting of TC and β-carotene content *versus* the colorimetric indices and photoacoustic spectroscopy signal

Technique	Colorimetry						PAS	
	L*	a*	b*	dE*	C*	h^0 (rad)	Xe-lamp (470 nm)	Ar-ion laser (481 nm)
TC by SP[a]	-0.165	0.981[c]	0.696	0.218	0.264	-0.254[b]	0.987[c]	0.991[c]
BC by HPLC[a]	-0.267	0.866[d]	0.419	0.022[b]	0.335	-0.295[b]	0.846[d]	0.859[d]

BC - □-carotene, assayed by HPLC; PAS – photoacoustic spectroscopy, TC - total carotenoids, assayed by spectrophotometry

[a]Fresh mass basis data.

[b]Low calibration sensitivity (slope).

[c] p <0.01.

[d] p <0.05.

Table 3. Analytical performance data for analysis of total carotenoids in apricots

Technique		Best linearity fit							Precision (RSD, %)		SEWM (%)
		TC range (mg/100 g)[a,b] N/*n*	*R*/ *RSS*[c]/ $s_{y/x}$[c]	Slope Mean	Slope RSD (%)	Intercept Mean	Intercept RSD (%)	*LOD* estimate (mg TC/100 g)[a]	Intrinsic	Measurement repeatability	
PAS (Xe, 470 nm)[d]		1.22-3.35 7/3	0.987[i]/ 1.112x10^{-4}/ 2.419x10^{-3}	1.36 V	5.3	0.074 mV	2.2	0.59	0.5-8.0[e] (av. 2.2)	1.5-2.7[f] (av. 1.9)	0.4-1.7
PAS (laser, 481 nm)[d]		1.22-3.35 7/3-4	0.991[i]/ 0.485/ 0.156	46.85 V	9.5	2.544 mV	3.9	1.10	0.1-0.6[e] (av. 0.3)	1.8-6.2[g] (av. 4.6)	0.1-0.3
Colorimetry	a*	1.22-3.35 7/3	0.981[i]/ 2.508/ 0.363	1.706 au x 10^5	6.3	12.795 au	1.9	0.70	-	0.4-2.9[h] (av 1.5)	-
	b*	1.22-3.35 7/3	0.696[j]/ 76.267/ 2.004	2.480 au x 10^5	24.0	29.529 au	4.6	2.67	-	0.4-1.9[h] (av. 1.0)	-

au – arbitrary unit, *LOD* (limit of detection) = 3.3$s_{y/x}$/slope, *N* – number of concentration levels, *n* - number of independent measurements at each concentration, PAS – photoacoustic spectroscopy, RSD – relative standard deviation, *R* – Pearson's correlation coefficient, *RSS* – residual sum of the squares, $s_{y/x}$ – residual standard deviation of the regression line, *SEWM* – standard error of the weighted mean, TC – total carotenoids.

[a] Fresh mass basis.

[b] Each sample analyzed by spectrophotometry (3 independent analyses).

[c] Number of observed pairs=21-22.

[d] Both Xe-lamp and Ar-ion laser PA signals expressed in mV.

[e] 512 successive readouts of the lock-in.

[f] *n*=3.

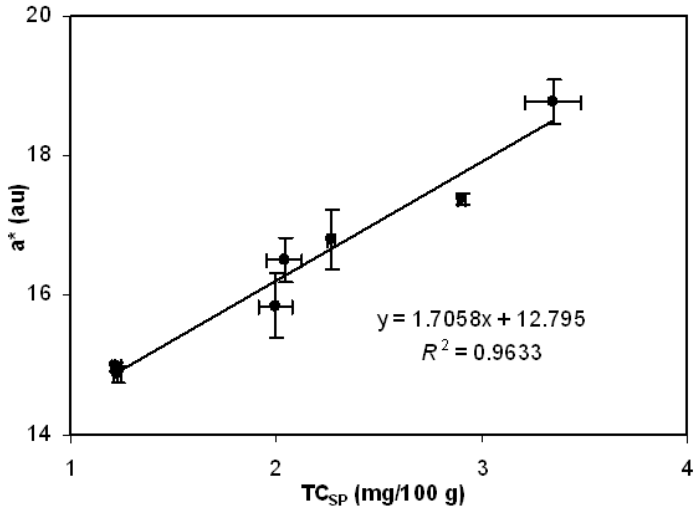
[g] *n*=3-4.

[h] Three loadings of the same, re-mixed material.

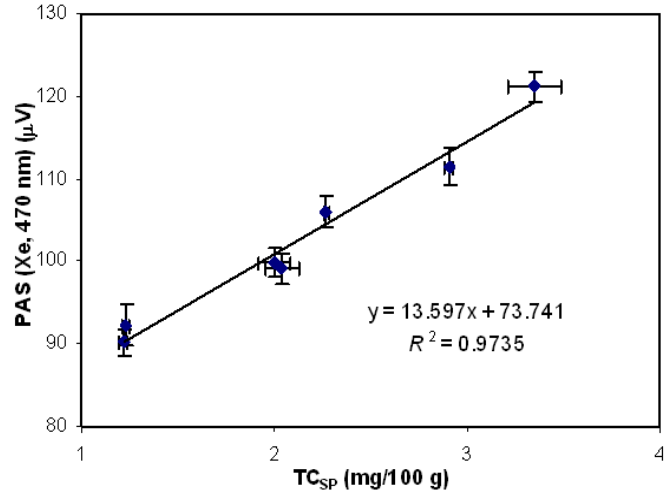
[i] Significant linear correlation, $p < 0.01$.

[j] Non-significant linear correlation.

a)



b)



c)

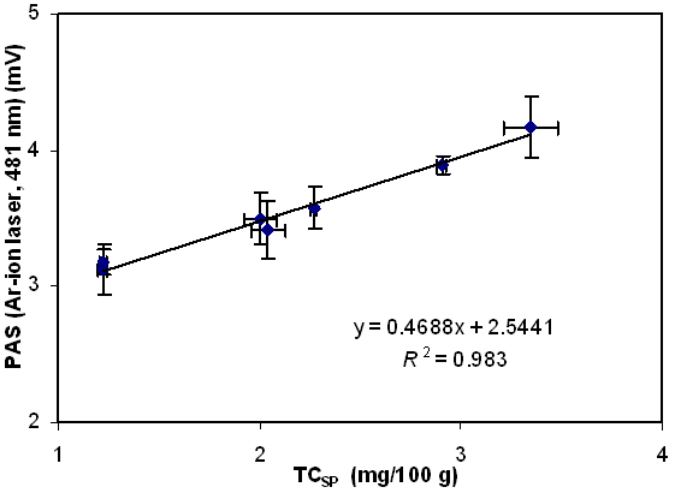


Fig. 1. Linear correlations between total carotenoids content (TC) (obtained by spectrophotometry, *n*=3) and: a) colorimetric index a* (*n*=3), b) Xe-lamp PAS (470 nm, 17 Hz, *n*=3), c) Ar-ion laser PAS (481 nm, 30 Hz, *n*=3-4). Each point stands for mean±SD.