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LYCOPENE DETERMINATION IN TOMATOES BY DIFFERENT SPECTRAL TECHNIQUES (UV-VIS, FTIR AND HPLC)

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Lycopene is one of the most important and useful carotenoids found in nature, and a potent antioxidant that has been shown to play critical role in cancer prevention. It were studied 26 types of tomato powder samples, using different analytical methods. UV-VIS spectrophotometry showed the absorption peaks of carotenoids from these tomato powder samples in order to identify the sample with the highest lycopene concentration. FTIR spectroscopy was used to quantify lycopene content in tomatoes varieties and showed a distinct vibration band at 957 cm-1 assigned to a trans CH deformation vibration of lycopene. The lycopene content accurately and reproducibly with a correlation coefficient of 0.9996. Results have been compared with HPLC chromatography. The outcome of the study shows that the FTIR method is a good analytical method for quantification of lycopene in tomatoes.

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Keywords: Lycopene, Carotenoids, FTIR spectroscopy

1. Introduction

Carotenoids, such as beta-caroten and lycopene, are important components of antioxidant defense against lipid peroxidation in living cells [1].

Lycopene, an aliphatic hydrocarbon, has received particular attention as a result of studies indicating that it has highly efficient antioxidant and free radical scavenging capacity [2]. This is the main reason that the development of tomato varieties with increased lycopene content requires efficient selection and the ability to measure lycopene in thousands of samples.

Fourier-transform infrared (FTIR) spectroscopy is a well established, nondestructive technique for analyzing agricultural and food products [3,4]. It offers much to the analyst because spectral bands may be assigned to specific chemical entities, and it provides bands arising from group vibrations with known assignment in most cases.

Attenuated total reflectance (ATR) as a sampling technique for FTIR measurements offers interesting possibilities for the analysis of liquid and solid foods.

Lycopene belongs to the family of carotenoids. It has a structure that consists of a long chain of conjugated double bonds, with two open end rings. The structure lycopene is the longest of all carotenoids. Lycopene ([C40H56], molecular weight 536.85) is an unsaturated hydrocarbon carotenoid containing 13 carbon-carbon double bonds, 11 of which are conjugated and arranged in a linear array. These conjugated double bonds are responsible for the vibrant red color of lycopene [5-7]. Lycopene is a lipophilic compound that is insoluble in water, but soluble in organic solvents, and it has a quenching constant double that of beta-carotene and 10 times alpha tocopherol (Hadley and others 2003). The quenching ability is directly related to the position of excited state energy levels, which depend on the length of the conjugated carbon double bone chain (Young and Lowe 2001).

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The objective of this study was to develop methodology for the rapid, accurate, and sensitive determination of lycopene in tomatoes using attenuated total reflectance Fourier-transform infrared (ATR-FTIR) spectroscopy, which is a reliable, accurate, simple and rapid technique for agriculture and food products [8].

2. Experimental

Materials and methods

1. Tomato samples

A total of 26 tomato powders were prepared from selected and sun-ripened tomatoes. Once skins have been removed, the tomatoes are concentrated and air spray dried at low temperature by spray drying technology which captures red color, pure taste and rich lycopene of ripe tomatoes.These samples represent genetically distinct varieties lines, and include a mix of commercial tomato samples from different processing industry. Samples were placed in 15 mL polypropilene containers, and stored at -20 ^{O}C until further analysis. A total of 88 spectra were used to test the predictive ability of the calibration model for lycopene content in tomatoes. Standards of lycopene was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO), and it was extracted in our laboratories after a literature method [9]. All solvents were certified LC or ACS grade (Fisher Scientific Co., Fairlawn, NJ).

2. Lycopene extraction

Lycopene from tomato products was extracted as follows: 5 g of sample (tomato powder) was homogenized in 50 ml methanol plus 1 g calcium bicarbonate and 5 g of celite [9]. The sample was then filtered through Whatman no.1 and no.42 filter papers. Lycopene was extracted using a sample of hexane: acetone (1:1, v/v), and quantified spectrophotometrically at 472 nm and expressed in mg/100 g FW.

3. Apparatus

UV-Vis. Spectral analysis has been done by using a SPECORD M400 spectrophotometer, with microprocessor and double beam. The wavelength range is between 185 - 900 nm.

Fourier transform IR spectroscopy (FT-IR). Standard spectra were collected by using a Perkin Elmer Spectrum GX spectrometer with a potassium bromide beam splitter and deuterated triglycine sulfate (DTGS) detector were used for all readings. The ATR accessory used a 3-reflection diamond crystal plate, providing a 3-fold increase in sample response compared to the standard single-reflection crystal plate (Pike Technologies). A range of 400–4000 scans were accumulated for each spectrum at a spectral resolution of 4 cm^{-1} and 32 scans. It was possible to use the drift accesory with the powdered pure substance, thereby allowing for a better and easier analysis. DRIFT spectra were recorded as Kubelka-Munk transformed spectra against a KBr background.

Attenuated Total Reflection Infrared Spectroscopy**.** The mid-infrared spectra were recorded in the range between 650 and 4000 cm^{-1} in a nine reflection configuration using a diamond-ZnSe ATR crystal. About 2-3 mg of tomato samples was placed on the surface of the ATR crystal (diameter, 0.5 mm^2). Three spectra for each sample were accumulated from 30 scans with a spectral resolution of 4 cm^{-1}. For further analyses, averaged spectra were used.

High performance liquid chromatography analysis. Lycopene in each tomato variety were analyzed by an HP 1050 reverse-phase HPLC system equipped with a photodiode array detector (Agilent Technologies, Palo Alto, CA). Dried lipid phase aliquots have been re-dissolved in a 3 ml methanol solvent. Sample (10 microl) has been injected in the HPLC system for carotenoid analysis.

A C30 YMC column (5microm particle size, 250x4.6 mm) (Waters Corp., Milford, MA) have been used in all separation.

Elution solvents used in these experiments are:
A: water
B: methanol
C: 86% acetonitril, 10% water, 4% formic acid;
D: 96% ethyl acetate, 4% water
Separations were carried aut with a 35 min linear gradient (10 % C + 90% D) at room temperature, 55 minutes linear gradient (100 % C) at room temperature. The flow-rate was 1.5 ml/min, with detection at 470 for lycopene, and the retention time was 69 min. The relative standard deviation of replicates (precision) was 5%.

3. Results and discussion

Lycopene, the red pigment of the tomato, is a C_{40}–carotenoid made up of eight isoprene units, Figure 1. In the figure 2 it is shows the ATR-IR spectrum of a dried tomato samples, indicating the signal from specific functional group vibrations.

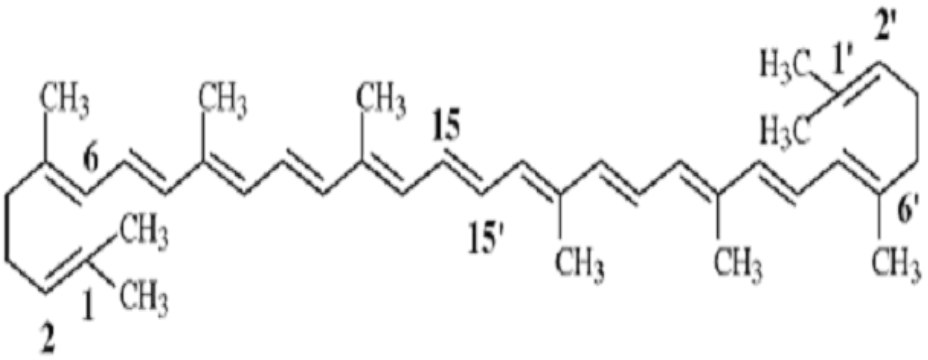

Fig. 1. The structure of lycopene

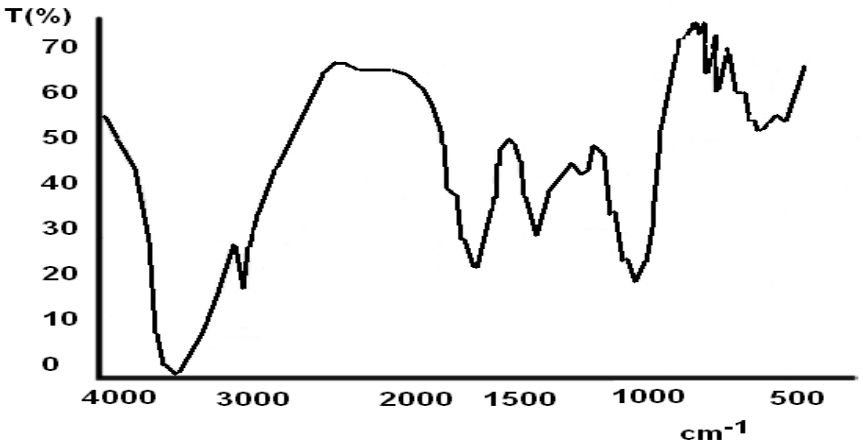

Fig. 2. FTIR spectra of tomato samples

The spectra show typical bands arising from amide (1650 and 1540 cm^{-1}) and lipid (1730-1765 [cm^{-1}] and 3000-2800 [cm^{-1}]) groups. Other bands occur at 1477-1400 [cm^{-1}] (C-H bending), 1100-1400 [cm^{-1}] (C-C and C-C-H stretching), and 1170-1115 [cm^{-1}] (C-O stretching). Strong and broad absorption bands of water are shown in the 3700-3000 [cm^{-1}] and 1600-1700 [cm^{-1}] range. The frequency region between 1200 and 900 [cm^{-1}] shows intense bands attributed to v(C-O-C)

vibrational modes of various carbohydrates and acids, which are abundant groups in tomatoes [10]. Frequencies of all types of deformations are found below 1000 [cm^{-1}] [11].

The second derivative transform (Figure 3) allowed for the extraction of useful band information through the removal of baseline variations and resolution of overlapping peaks [12,13,14].

The spectral signal obtained at a frequency of 957 [cm^{-1}] can be attributed to the presence of trans CH out-of-plane deformation vibration [15-17] of lycopene (Figure 4) found in tomatoes. The lycopene content of the tomato ranged around 8-9 micog/mg of dried matter based on the HPLC analysis, values within those reported in the literature [16].

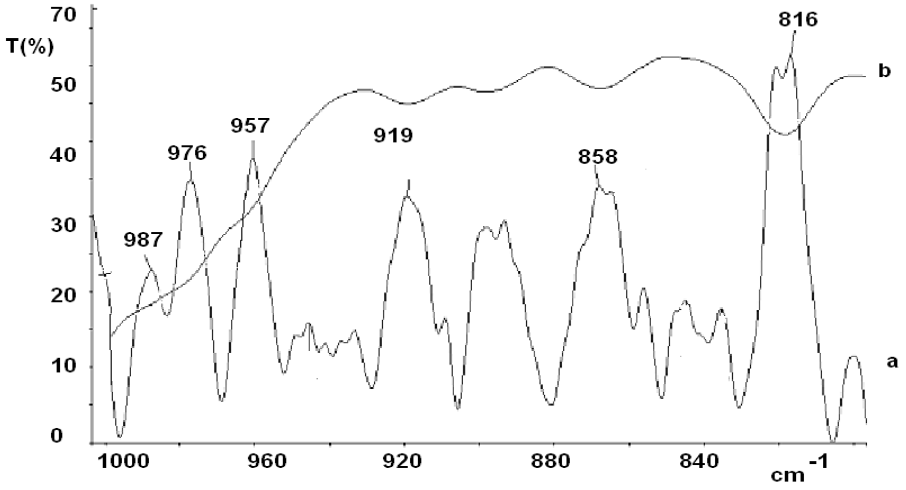

Fig. 3. The second derivative (a) and the FTIR spectra (b) of the sample with highest lycopene concentration (8.63 microg/mg DW).

Comparison of the second derivative spectra of tomato samples that included lycopene-containing showed increased IR absorbance bands in the 850-1200 [cm^{-1}] region that correlated with the increased lycopene content of tomatoes.

The etalonation curve deduced from HPLC measurements is shown in Figure 4. The calculated detection limit (LD) is 0.17 microg/l for lycopene, and the quantification limit (LQ) for lycopene is 0.6 microg/l.

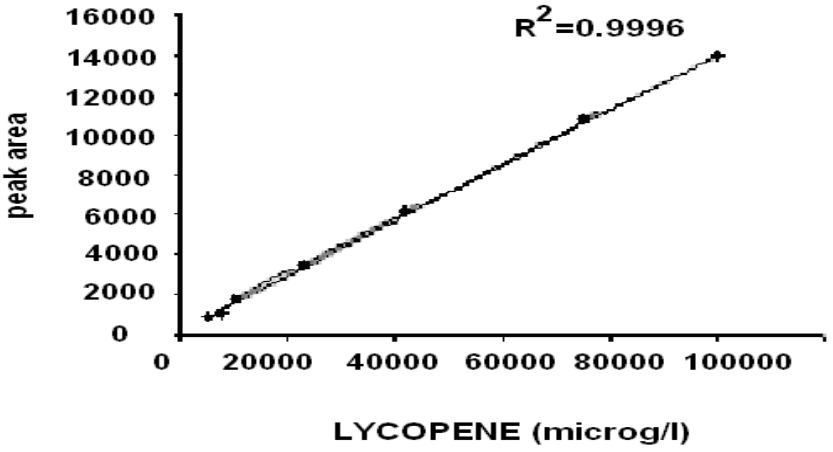

Fig. 4. Calibration curve for lycopene by HPLC

The ATR-FTIR spectra extracted important absorption features in the fingerprint region (1200-900 [cm^{-1}]) with absorption bands due to CH-deformation vibrations (957 [cm^{-1}]) largely influencing the spectral variation [15-18]. The correlation plots between the obtained results of lycopene determination in different tomatoes samples using proposed FTIR and standard HPLC methods are presented in Figure 5. A relatively high correlation coefficient of R^2 = 0.9996 for lycopene determinations in all studied tomatoe samples indicates a good agreement between both methods.

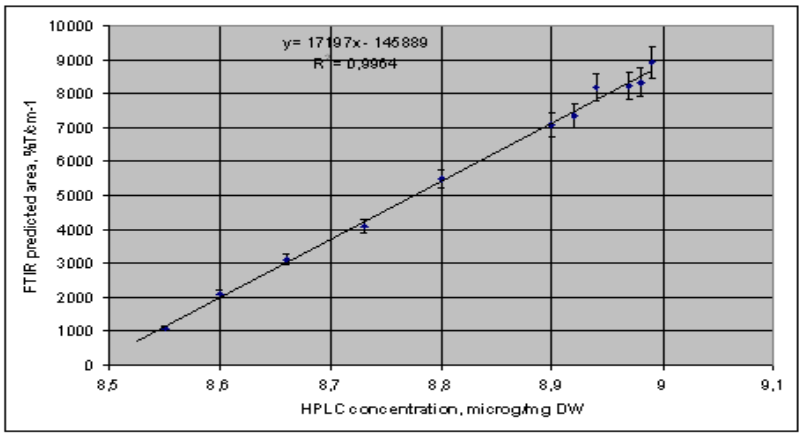

Fig. 5. The correlation plots between the obtained results of lycopene determination in different samples using proposed FTIR and standard HPLC methods

The retention time for lycopene detected by HPLC has a specific value both in standard and in powder tomatoes, as it is shown in Figure 6.

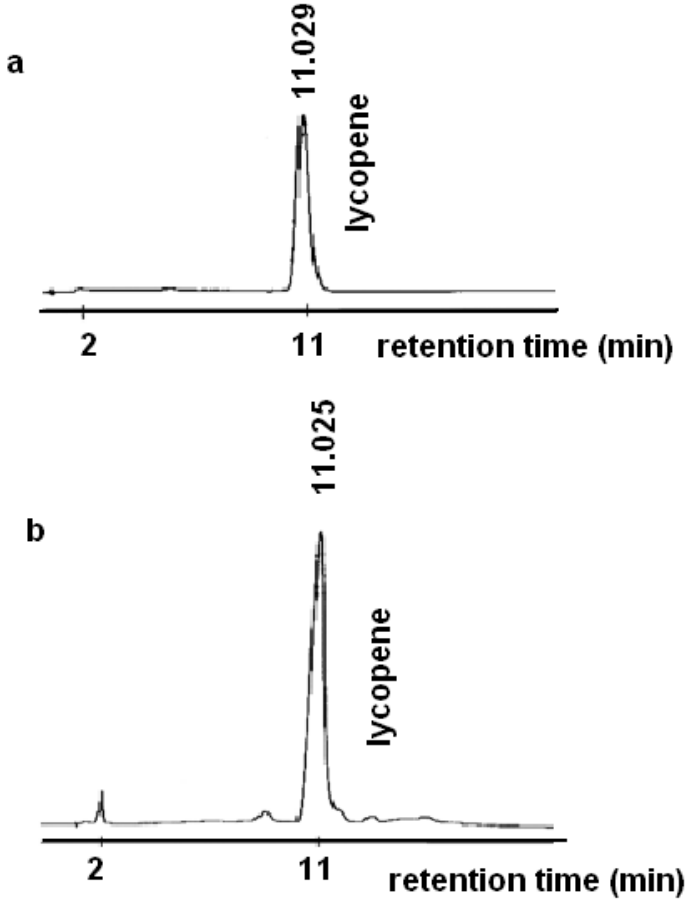

Fig. 6. Retention time from HPLC of lycopene standard (a) and tomato powder (b)

By using both analytical techniques, was possible to determine lycopene from tomatoes powder samples. Table 1 showed the average predicted IR lycopene content from 12 tomato breeding lines as compared to the HPLC reference values. Therefore, the comparison between the results obtained by standard HPLC and the proposed FTIR methods indicates that the two procedures give statistically comparable values of lycopene concentration in tomato samples.
The accuracy of the proposed and standard methods was good.

Table 1. Average predicted IR lycopene values compared to average HPLC reference values

Sample no.	Name	ATR µg/mg DW	HPLC µg/mg DW
0	Olka	8.55	8.57
1	Tukas	8.56	8.55
2	Tamek	8.55	8.49
3	Tat	8.63	8.77
4	Akfa	8.59	8.6
5	Burcu	8.6	8.62
6	Cappy	8.56	8.6
7	Dimes	8.59	8.64
8	Metro	8.55	8.62
9	Dunya	8.56	8.7
10	Migros	8.55	8.65
11	Sundried Vacuum packed whole	8.55	8.6
12	Yedi	8.58	8.67

UV-VIS spectrum of lycopene shows four peaks located at 420 nm, 444 nm, 470 nm and 500 nm (figure 7). Samples were studied and analyzed in terms of content of chlorophylls, in this sense is recorded spectra for products as such and after saponification water extraction in petroleum ether acetone system. It noted the absence of absorption maximum wavelength at 620 nm, which special for chlorophylls.

Regarding the image in visible absorption spectra for all samples analyzed is similar. The four maximum absorptions are present in all 26 samples. What makes the difference between them is the ratio of the maximum absorption is closely related to the composition of carotenoid extracts.

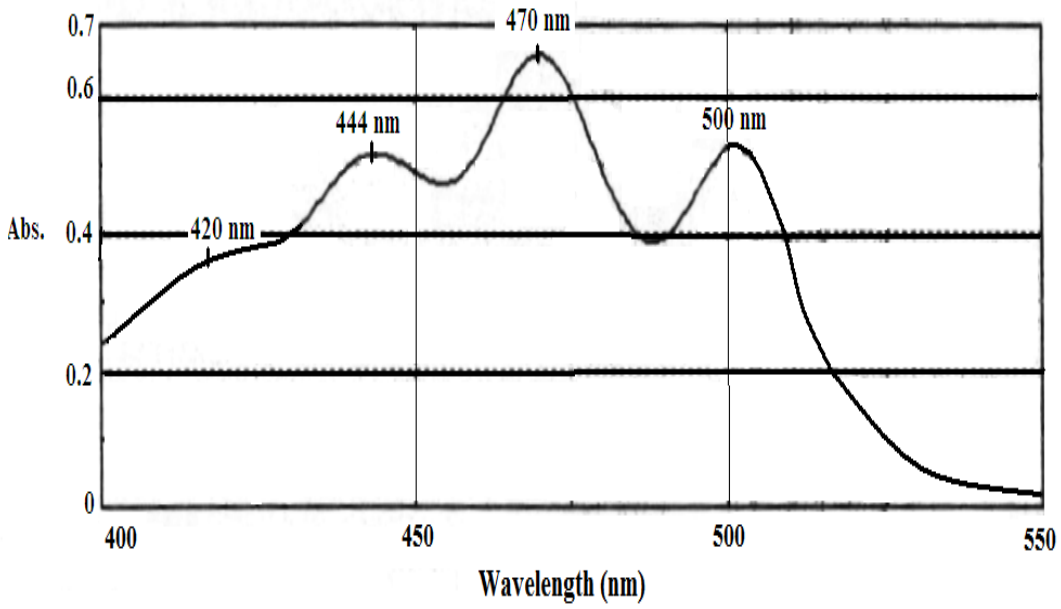

Fig. 7. UV-Vis spectrum of extract tomato sample

By analyzing the absorption spectra of pure compounds presented in the literature [19], it is found that alpha-carotene and lutein were the most important contribution in the absorption spectrum bands at 420 nm and 444 nm. Lycopene is the most important contribution to the absorption band at 470 nm and 500 nm.

It was calculated the total content of carotenoids in extracts of tomato mixture, and the representation is showed in table 2.

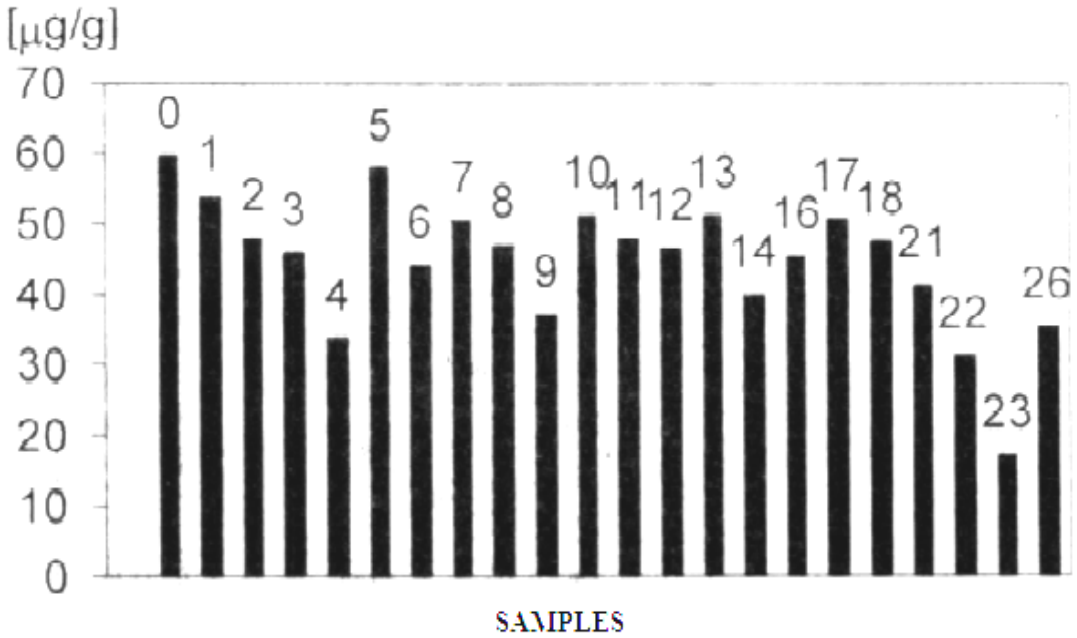

Table 2. Total content of carotenoids in extracts of tomato mixture.

4.Conclusions

A simple FTIR protocol allowed for the rapid, accurate, sensitive, and reliable determination of lycopene in tomatoes with minimal sample preparation. Using transformed IR spectral data (second derivative, 5-point window), the lycopene content in tomatoes involves a

correlation between FTIR predictions and HPLC reference method. Application of the FTIR technique to more homogeneous processed tomato products may improve the predictive ability of the multivariate models. A distinct CH out-of-plane deformation vibration of a C-C double bond marker peak was found in trans-lycopene-containing tomatoes.

Acknowledgments

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