

# Spectrophotometric Analysis of Lycopene in Tomatoes and Watermelons: A Practical Class

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**Abstract:** Lycopene is one of the most important and abundant carotenoids. It has been shown to play a very important role in human nutrition, mainly due to its high antioxidant activity. In order to show our students of analytical chemistry a practical application of food analysis as well as the different steps of the analytical methodology, we have carried out a practical analytical chemistry class which consists on the determination of lycopene in both tomato and watermelon samples by means of a quick spectrophotometric method with data analysis. The proposed class, which will be described (as well as the results obtained by our students), can be applied in subjects related with analytical chemistry, food analysis, agriculture, etc.

## Introduction

Most of the orange, yellow and red colors of leaves, fruits and flowers are due to carotenoids. These essential nutrients in the human diet are thought to provide health benefits by decreasing the risk of various diseases, particularly certain cancers, cardiovascular and eye diseases [1]. One of the most important and abundant carotenoids is lycopene (Figure 1), an open chain unsaturated carotenoid containing thirteen double bonds (eleven conjugated and two non-conjugated double bonds). It is chiefly found in tomatoes, apricots, ping grapefruit, guava and watermelon [2], being tomatoes and tomato derived products the richest source of lycopene in the diet (tomatoes are also one of the largest vegetables crops in the world in terms of production). The biological activity of lycopene includes a very important antioxidant activity (protective effects on certain types of cancers have also been described) as well as the induction of cell communication and modulation of hormonal, immune systems and other metabolic pathways [3]. As a result, the determination of lycopene content in food samples is of a relevant importance, providing very important information from the nutritional and physiological point of view.

Nowadays, new analytical chemistry practical classes should provide students a global view of the different fields in which this discipline plays an important role in daily life, such as pharmaceutical, environmental or food analysis, quality control, etc. Among them, food analysis and, moreover, the determination of important nutrients, such as vitamins and antioxidants is of special importance because of their important nutritional value in the human diet. Not very often, practical classes dealing with the analysis of antioxidants are carried out at the university level, mainly due to the complexity of sample treatments and costly instrumentation. As a result, and based on recent research papers, which have suggested the analysis of lycopene in carotenoid-rich samples by using a simple and rapid spectrophotometric method after extraction of the samples with hexane [4, 5, 6], we propose a practical class consisting in the quick spectrophotometric quantification of

lycopene in different tomato and watermelon samples, as well as the analysis and comparison of the obtained data. Results obtained by the students are also shown and commented.

## Experimental

**Chemicals.** A standard solution of lycopene in hexane (0.04 mg/mL) was prepared, wrapped in foil, and stored in the dark at 4°C. Working mixtures of pertinent concentrations can be made by appropriate combination and dilution with hexane. The conjugated-double-bond system of carotenoids, in general, produces the main problems with their manipulation (they are unstable to light, oxygen, heat, acid, and alkaline conditions). That is why particular attention should be paid in the sample/standard preparation, manipulation and storage. Butylated hydroxytoluene (BHT), acetone, hexane and ethanol were purchased from Merck (Darmstadt, Germany). Milli-Q water was also used (deionized by using a Milli-Q system, Millipore, Bedford, MA, USA).

**Safety.** Safety goggles must be worn when manipulating chemicals and solvents. Ensure all solvent residues are disposed in appropriate residue containers.

**Equipment.** These experiments requires a UV-visible spectrophotometer. In our case we have used the HP8453 UV-visible spectrophotometer from Hewlett Packard controlled by an HP Vectra XA 5 computer (Pentium IV) with ChemStation Software. Detection was carried out at 503 nm.

**Samples.** Watermelon and tomato samples were bought in a local supermarket. Special care was taken to select the most mature samples.

**Extraction Method.** Extraction method was performed according to Fish et al. [4]. Samples were first chopped and homogenized in a laboratory homogenizer. Approximately 0.3 to 0.6 g samples were weighed and 5 mL of 0.05% (w/v) BHT in acetone, 5 mL of ethanol and 10 mL of hexane were added. The recipient was introduced in ice and stirred on a magnetic stirring plate for 15 min. After shaking, 3 mL of deionized water were added to each vial and the samples were shaken for 5 min on ice. Samples were then left at room temperature for 5 min to allow the separation of both phases. The absorbance of the hexane layer (upper layer) was measured in a 1-cm-path-length quartz cuvette at 503 nm blanked with hexane.

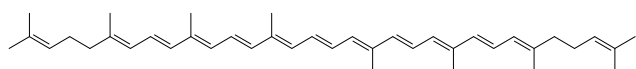


Figure 1. Chemical structure of lycopene.

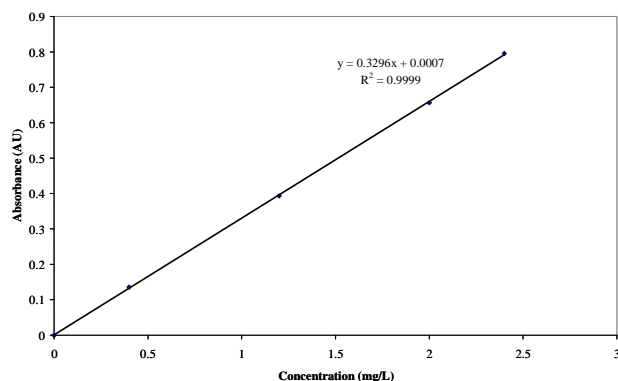


Figure 2. Absorbance versus lycopene concentration (mg/L) in hexane.

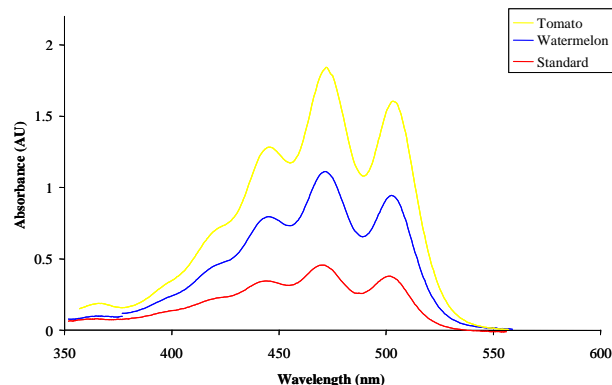


Figure 3. Spectra of a standard mixture of lycopene in hexane and hexane extracts of two of the analyzed samples (a watermelon and a tomato).

**Statistical Program.** For data treatment and evaluation the statistical package Statistica 6.0 from StatSoft Inc. (Tulsa, OK, USA) was used.

## Results and Discussion

Based on different published research works that can be found in the scientific literature [4, 5, 6], we have carried out with our students of analytical chemistry a practical class that consists on the determination of lycopene in different types of samples by using a very simple spectrophotometric method as well as the statistical analysis of the results. For this purpose, the method described in the experimental section and taken from [4] was followed. The proposed method uses a much lower amount of organic solvents than conventional procedures. According to this method, absorbance at 503 nm ( $A_{503}$ ) has been selected to avoid interferences from other carotenoids present in the samples although the absorbance at this wavelength value is not the absorbance of the greatest of lycopene in hexane.

Several solutions of lycopene in *n*-hexane at different concentrations were prepared and absorbance was measured at 503 nm (concentrations tested can be in the range between 0

and 3 mg/L) after suitable calibration of the instrument with hexane. By appropriate plotting of the absorbance versus the concentration of lycopene the calibration graph shown in Figure 2 was obtained.

A good linearity with correlation coefficients of 0.9999 was obtained. After the calibration curve was obtained, the method described in the experimental section was applied to the determination of lycopene in both tomato and watermelon samples. The content of lycopene in the samples can be estimated by two methods. One of them (a theoretical method) makes use of the molar extinction coefficient of lycopene in hexane ( $17.2 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ ) determined in the literature [8]. In this case, the Lambert–Beer law can be described as:

$$\text{Absorbance at 503 nm } (A_{503}) = \epsilon(\text{M}^{-1} \cdot \text{cm}^{-1}) \cdot b(\text{cm}) \cdot [\text{Lycopene concentration (M)}]$$

By properly substituting the molar extinction coefficient of lycopene in hexane ( $17.2 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ ) as well as the molecular weight (536.9 g) and by changing the units, the final equation will be

$$\text{Lycopene content (mg/kg)} = A_{503} \times 31.2/\text{g tissue}$$

While one method is based on the experimental data obtained by the students (calibration curve). In our case, from Figure 2 (and by appropriate substitution in the Lambert–Beer law equation) it can be found that

$$\text{Lycopene content (mg/kg)} = (A_{503} - 0.0007) \times 30.3/\text{g tissue}$$

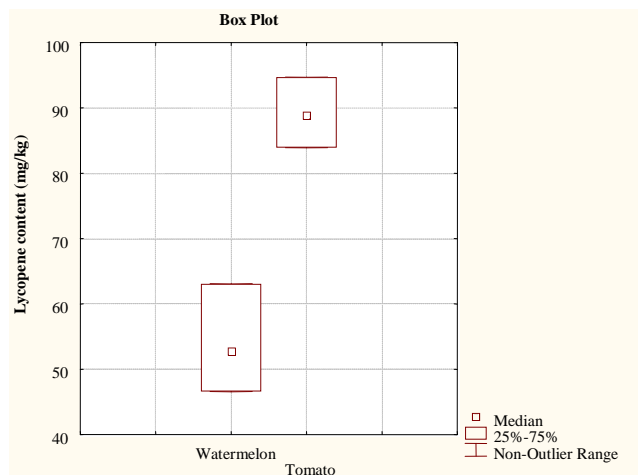
The use of these two formulas in which the lycopene content is given in mg/kg is very useful to evaluate the lycopene content and to compare the data with those of the literature.

The lycopene content of each sample was then estimated using the absorbance measured at 503 nm and the sample weight. The proposed method has been shown to provide reasonable results for those foods in which lycopene constitutes at least 70% of the constituent carotenoids as, for instance, in tomatoes and watermelons; that is why the method was applied to the analysis of these samples. Figure 3 shows the absorbance spectra (in the wavelength region of interest, i.e. 503 nm) of a standard mixture containing 1.25 mg/L of lycopene in hexane as well as the hexane extracts of two of these samples (one corresponds to a watermelon sample and the other one to a tomato sample). As it can clearly be observed the spectra of both extracts and standards are very similar.

Table 1 shows the lycopene content of the analyzed samples by the use of both experimental and theoretical data. Good agreement for both was achieved with a relative error below 3%. Also, the content of lycopene determined by the students is very similar to those indicated in the literature for analogous samples [4, 5]. As an example, see for lycopene content in watermelons the works by Fish et al. [4] or Perkins-Veazie et al. [5] and for tomatoes the work of Markovic et al. [7]. Concerning the analysis of lycopene in watermelons, it has been stated in the literature that lycopene content varies widely among cultivators and with production season [5]. A comparison of the results we obtained with the data described in the literature as well as with methods developed by other researchers shows the students the need for an appropriate

**Table 1.** Lycopene Content Calculated by the Students, Using Both Experimental and Theoretical Data.

Samples	Lycopene content (mg/kg) using experimental data (fresh weight)	Lycopene content (mg/kg) using theoretical data (fresh weight)	Relative error (%)
Watermelon 1	63.1	64.9	2.77
Watermelon 2	46.6	48.0	2.91
Watermelon 3	52.9	54.4	2.76
Tomato 1	89.0	91.5	2.73
Tomato 2	84.0	86.4	2.78
Tomato 3	94.7	97.5	2.87

**Figure 4.** Box and whiskers plot of the content of lycopene in watermelons and tomatoes.

comparison of their results. As a result, another interesting practical approach for other practical classes could be the analysis of local and foreign watermelons, even the analysis of different varieties of watermelons or of watermelons at a different stage of maturation and a comparison to the data described in the literature. The same could also be done for tomato samples.

After the determination of lycopene content was carried out, we developed, as an introductory approach to data analysis, a box and whiskers plot (even with such a low number of samples) in order to show the students the utility of this tool for sample comparison. We introduced at this point the theoretical explanation of the plot tool. They could easily observe that the data concerning the content of lycopene in tomatoes is higher than that of watermelon samples and also that there is higher dispersion of the data in watermelon

samples. The median could also be easily observed. The proposed method can be of a higher utility if the number and types of samples is high, which could be done if the class is large enough and different samples are analyzed by different groups of students. In this particular case, the presence of outliers can not be detected because the number of samples analyzed is very low, but with a higher number of samples it would easily show students a method for detecting data that does not follow normal behavior and, thus, show them that a study of the history of the sample or a revision of the analytical procedure for that specific samples is necessary. The application of these chemometric tools shows the students the need and advantages of statistical data treatment and that their analysis does not end with their numerical data.

## Conclusions

We describe here, as a practical laboratory class developed with our analytical chemistry students, the fast determination of lycopene content in tomatoes and watermelons by means of a spectrophotometric method. Because the proposed method is relatively fast and requires low quantities of organic solvents (much lower than other procedures for the conventional extraction of lycopene) it is recommend for practical analytical chemistry classes. Students will learn a practical analytical methodology. The class can also serve as an introduction to data treatment or chemometric analysis.

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