ISSN: 2226-7522(Print) and 2305-3327 (Online)
Science, Technology and Arts Research Journal
July-Sep 2012, 1(3): 17-23
www.starjournal.org


Original Research

# Visible/Near Infrared Spectroscopic Method for the Prediction of Lycopene in Tomato (*Lycopersicon esculentum,* Mill.) Fruits

**Wondu Garoma Berra**

Food Science and Bioprocess Technology Institute, Wollega University, Post Box No: 395, Nekemte, Ethiopia

## Abstract

The aim of the present study was to predict the potential of visible and near infrared (Vis/NIR) Spectroscopy in estimating the amount of lycopene in intact tomato. Eight tomato varieties from loose and cluster type were selected and harvested at commercial ripening stage for the study. The tomato cultivars were prepared after 3 days storage at 18°C and 80% Relative Humidity (RH) before they were subjected to visible and near infrared (Vis/NIR) spectroscopy measurements. For the visible and near infrared (Vis/NIR) spectroscopy measurements four tomato fruits from each variety were prepared as appropriate by separating of clusters, removal of attached stems and assigning a number to each of the tomato fruits. Accordingly, the spectral measurements were done on intact tomato fruits. The visible and near infrared (Vis/NIR) spectroscopy combined with multivariate calibration method such as principal component analysis (PCA) and partial least-squares (PLS) has showed a reasonable prediction performance. The established PLS model at full spectral range (380 – 1700 nm) was with correlation coefficient *(r)* =0.90 and standard error of prediction (SEP) = 0.65 mg/100g for lycopene. However, the best model for prediction was obtained when the spectral range was limited to the visible range (380 – 950 nm), correlation coefficient *(r)* = 0.92 and standard error of prediction (SEP) = 0.58 mg/100g. Although HPLC is the most accepted method of analysis, Vis/NIR spectroscopy measurement combined with multivariate techniques has the potential to estimate lycopene in intact tomato fruits.

**Article Information**

**Article History:**

**Received** : 18-07-2012

**Revised** : 16-09-2012

**Accepted** : 20-09-2012

**Keywords:**

Tomato
Visible and near infrared
Lycopene
Loose
Cluster

***Corresponding Author:**

**Wondu Garoma Berra**

**E-mail:**

wondu_2005@yahoo.com

wondugaroma@gmail.com

## INTRODUCTION

Lycopene is the most abundant carotenoid pigment principally responsible for the characteristic deep red color of ripe tomato fruits and tomato products. Recently, a lot of attention has been paid to its biological and physicochemical properties, especially related to its effect as a natural antioxidant (Anantha narayan and Choudhari, 2007; Barba *et al.,* 2006; Qiu *et al.,* 2006; Rao, 2007; Maguer and Shi, 2000), with a singlet oxygen physical quenching rate constant almost twice as high as that of β-carotene and more than ten times higher than that of α-tocopherol (Di Mascio *et al.,* 1989; Ali and Rao, 2007; Pol *et al.,* 2004; Shi *et al.,* 2003).

A number of analytical procedures have been developed and applied for the determination of antioxidants in fresh tomato and tomato products (Gomez-Romero *et al.,* 2007) in a destructive and non-destructive way including electronic tongue (Rudnitskaya *et al.,* 2001), colorimeter, electronic nose (Berna *et al.,* 2002), acoustic firmness sensor (AFS), near infrared spectroscopy (NIR) (Peirs *et al.,* 2003; Yong *et al.,* 2005; Baranska *et al.,* 2006), nuclear magnetic resonance (NMR) (Gladdeny and Alexanderz, 1996), NIR-FT-Raman spectroscopy (Schulz *et al.,* 2005), Fourier transform infrared spectroscopy (FTIR), hyperspectral imaging (Berna, 2006), etc.

However, the analytical methods used for lycopene are rather complicated (Barba *et al.,* 2006), and not all analytical methods available for carotenoid analysis in food products are suitable for lycopene rich foods due to its low solubility in some of the solvents employed, as in the case of methanol and due to the fact that use of other

solvents may interfere with the HPLC mobile phase applied for carotenoid separation.

Furthermore, the instability of lycopene during processes of extraction, handling, and elimination of organic solvents makes the sample preparation for lycopene an extremely delicate task, often requiring successive and complex procedures to ensure that all the carotenoids are extracted (Barba *et al.,* 2006; Maguer and Shi, 2000; Xu *et al.,* 2006).

Therefore, evaluation of the potential of the non-destructive Vis/NIR reflectance spectroscopy in determination of lycopene content in tomato fruits was importantly considered in this study.

## MATERIALS AND METHODS

### Experimental Design and Plant Materials

A total of eight tomato varieties, all harvested at commercial ripening stage were selected for the study and prepared after 3 days storage at 18 °C and 80% RH before subjected to Vis/NIR spectroscopy measurements The tomato varieties included were four loose tomato cultivars (Growdena, Brodena, DRW 75-93 and Excelsior) and four cluster types (Tricia, Clotilde, Bonaparte and Plaisance). For the Vis/NIR Spectroscopy measurements four tomato fruits from each cultivar were prepared as appropriate by separating of clusters, removal of attached stems and assigning a number to each of the tomato fruits. The spectral measurements were done on intact tomato fruits. Finally, HPLC analyses were carried out on same fruits for comparison.

### Vis/NIR Spectroscopy Instrumentation

The spectra were acquired in the wavelength range between 380 nm and 1700 nm. The spectrophotometer (Corona Fibre VISNIR 1104-400, ZEISS, Germany) worked with a single-beam diode array (polychromator) and two sensors (Si, Hamamatsu S 3904 (380-950 nm, 3 nm/diode) and an In Gas array (950-1700 nm, 6nm/diode)). The light source for Vis/NIR (380-1700 nm) could be used both in the visible and near infrared region. The light source consisted of a 9W, 5V stabilized halogen lamp which was directed to the fruit, placed on a black holder (Figure 1). The reflected light was captured by bundled detecting fibers under an angle of 45 degrees to avoid specular reflection and was guided towards the grating polychromator. The light source and spectrophotometer were switched on one hour prior to the acquisition of the spectra.

A reflectance spectrum was taken at approximately two equidistant positions on the equator of each tomato with the help of CORA software for windows 98/NT 4.0/2000. The spectra acquired on the spectrophotometer were corrected for the dark current response of the detector. Each reflectance spectrum was divided by a white reference spectrum recorded on a $BaSO_4$ disc. Furthermore, each measured spectrum was the average of 100 individual optical scans with 2 nm increments. For further calculations the average spectrum of the two sides, which means a total of 200 scans was used for data processing.

### Statistical Analysis

Multivariate techniques (PCA and PLS) were also used to analyze the NIR spectra and correlate the NIR spectra with the HPLC reference measurements. The Unscramble version 9 software (CAMO AS, Trondheim, Norway) was used to build multivariate calibration models based on partial least-squares (PLS). The calibration models were validated by means of cross validation, where a small set of randomly selected observations (leave-one-out) was left out to construct the model and is used afterward for validation purposes. This procedure was repeated until all samples have been used for validation. The accuracy of PLS calibration models was calculated as the standard error of prediction (SEP).

$$SEP = \sqrt{\frac{1}{Ip-1}\sum_{i=1}^{Ip}(Yi - yi - bias)^2}$$

$$bias = \frac{1}{IP}\sum_{i=1}^{Ip}(Yi - yi)$$

**Where,**

$Y_i$ is the predicted value of the $i^{th}$ observation,
$y_i$ is the measured value of the $i^{th}$ observation &
$I_p$ is the number of observations in the validation.

## RESULTS AND DISCUSSION

In this study the use of Visible/Near infrared reflectance spectroscopy (Vis/NIR) was evaluated for predicting the lycopene concentration of tomatoes. The same tomato

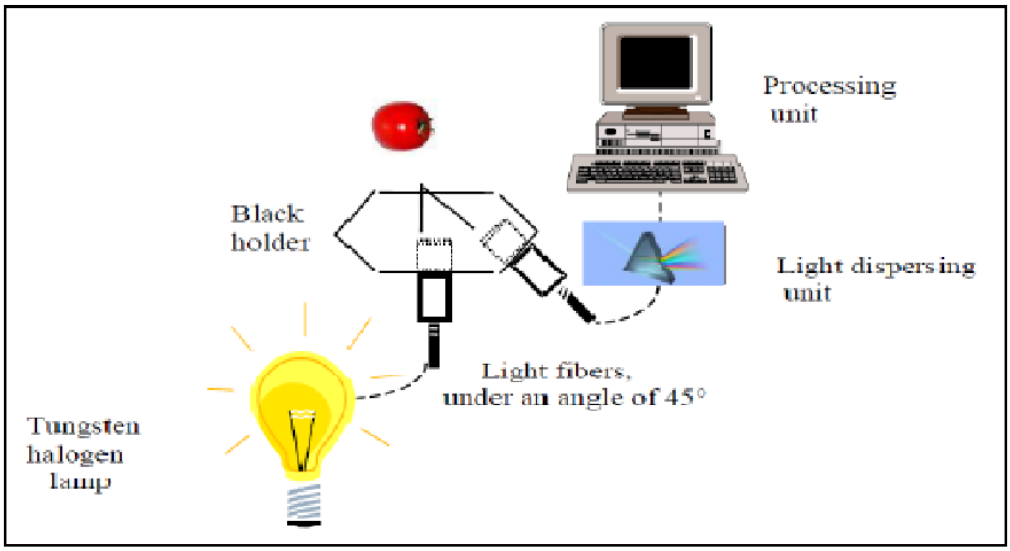


**Figure 1:** Schematic design of the NIR diode array spectrometer showing component parts.

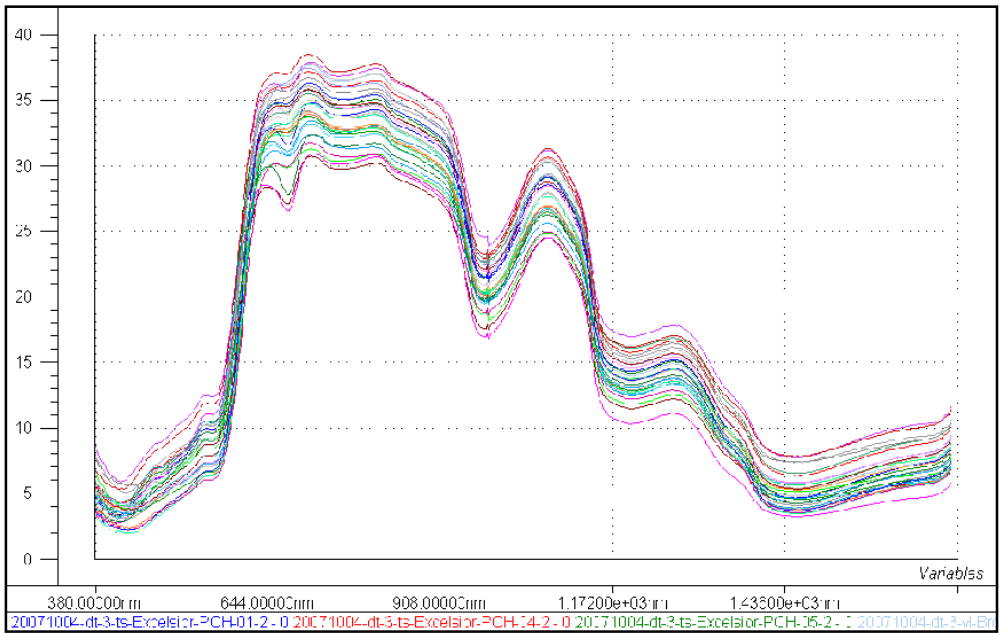


**Figure 2:** Averaged spectral data for the different cultivars (*Tricia, Bonaparte, Clotilde, Brodena, Growdena, DRW 75 93, Excelsior and Plaisance*).

cultivars analyzed by Vis/NIR spectroscopy were measured by HPLC for comparison. The objectives were to establish relationships between non-destructive Vis/NIR spectral predictions of lycopene and HPLC measured values of lycopene. Intact tomato fruits were measured by reflectance in a spectral range of 380–1700nm. The average spectrum (relative reflectance vs. wavelength) was given for each cultivar (Figure 2). A large diversity between the spectra of the cultivars was observed as clearly shown in the Figure 2.

As it can be observed from Figure 2, there are differences in reflectance between cultivars for the whole spectral range but a remarkable difference is noticed between 644 nm and 908 nm. To see more clearly differences between spectra, a principal component analysis (PCA) was performed. The calibration models are developed by partial least square regression

(PLSR) using quantitative measures of lycopene concentration as a reference method. Calibration models were set separately at three wavelength ranges: the visible (380–950 nm), NIR (950–1700 nm) and full spectrum (Vis/NIR) i.e. 380–1700 nm. Cross-validation was used for validating the models.

The performance of the calibration model in predicting lycopene content of tomato cultivars was quantified by the standard error of prediction (SEP), the root mean squared error of prediction (RMSEP) and the calibration coefficient (*r*) between the predicted and measured parameters. Good models should have low SEP and high correlation coefficients. The systematic difference between predicted and measured (*bias*) is also indicative for the performance quality of calibration model. The correct number of latent variables for the PLS model was determined based on the minimum residual validation variance.

The average lycopene content of tomato cultivars (Table 1) ranged from 2.5 to 7 mg/100 g (fresh weight basis). This result was in closest values (2.62–6.29 mg/100g fresh weight) which were reported by Baranska *et al*. (2006) for tomato fruit and various tomato products. However, the mean range obtained in this particular study was relatively lower when compared with those reported (6 to 13 mg/100g wet basis) for tomato breeding selections by Halim *et al*. (2006).

The coefficient of variation (RSD) for replicated HPLC assays was varying between 5.4 to 27.8% for fresh tomato samples depending on cultivar. Although higher value RSD of HPLC measured values seems to have an impact in the uncertainty of the PLS prediction, the overall result shows comparable estimation of lycopene content in tomatoes among the analytical techniques.

**Table 1:** Cross-validation results using PLS models for lycopene content in tomato cultivars in the visible, NIR and full wavelength range.

| | Element | Slope | Offset | Correlation *(r)* | RMSEP | SEP | Bias |
|---|---|---|---|---|---|---|---|
| **VIS/NIR** | 30 | 0.833140 | 0.741082 | 0.898817 | 0.636604 | 0.647378 | - 0.011675 |
| **VIS** | 30 | 0.879727 | 0.531589 | 0.920993 | 0.566834 | 0.576416 | - 0.011001 |
| **NIR** | 28 | 0.687015 | 1.436127 | 0.816758 | 0.866122 | 0.881541 | 0.028419 |

**Table 2**: Predicted Vis/NIR lycopene values compared to HPLC reference measured values.

| Cultivars | HPLC (mg/100mL) | RSD* (%) | Predicted values (mg/100g fresh weight basis) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Vis/NIR | RSD[a] | Vis | RSD[a] | NIR | RSD[a] |
| **Tricia** | 5.20 | 27.83 | 4.69 | 25.08 | 4.79 | 25.56 | 4.82 | 11.14 |
| **Clotilde** | 5.58 | 5.37 | 5.87 | 8.97 | 5.74 | 13.26 | 6.54 | 8.88 |
| **Bonaparte** | 6.64 | 22.38 | 6.01 | 12.81 | 6.27 | 15.62 | 5.17 | 21.59 |
| **Plaisance** | 5.02 | 13.28 | 5.27 | 14.24 | 5.10 | 17.08 | ** | ** |
| **Growdena** | 3.77 | 15.26 | 3.86 | 27.37 | 3.98 | 21.51 | 3.80 | 18.70 |
| **Brodena** | 2.81 | 27.31 | 3.21 | 47.68 | 3.14 | 32.89 | 3.14 | 26.22 |
| **DRW 7593** | 3.56 | 13.90 | 3.73 | 21.36 | 3.58 | 32.87 | 4.01 | 9.62 |
| **Excelsior** | 3.22 | 24.45 | 3.25 | 7.29 | 3.25 | 16.94 | 3.56 | 8.14 |

** Not predicted in the NIR range (Outliers), * Relative Standard Deviation in percentage

The variability in lycopene content between the cultivars was analyzed by the principal component analysis (PCA) based on the visible, NIR and full spectral ranges. The score plot based on concentration in the visible range which is presented in Figure 3 showed good separation of cluster and loose tomatoes. The first two principal components PC1 and PC2 explained 71% and 23% of the total variance respectively. Conversely, the score plot based on NIR spectral ranges shows no well separation of the two tomato types as presented in Figure 4. Based on the NIR spectral ranges PC1 and PC2 explained 92% and 7% of the total variance. In this study the score plot based on the full spectral ranges were also analyzed and the first two principal

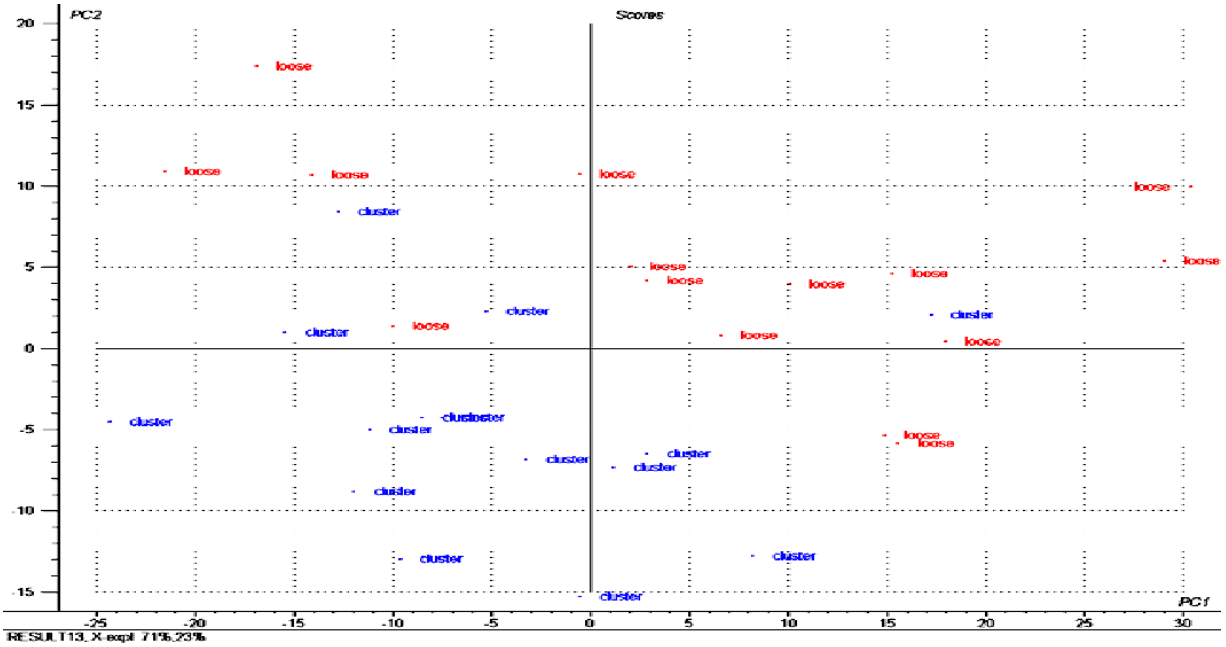


**Figure 3:** The scoreplot (PCA analysis) of the spectra of eight different tomato cultivars (loose and cluster) in the visible spectral range (PC1 and PC2 explain 71% and 23% of the variability respectively).

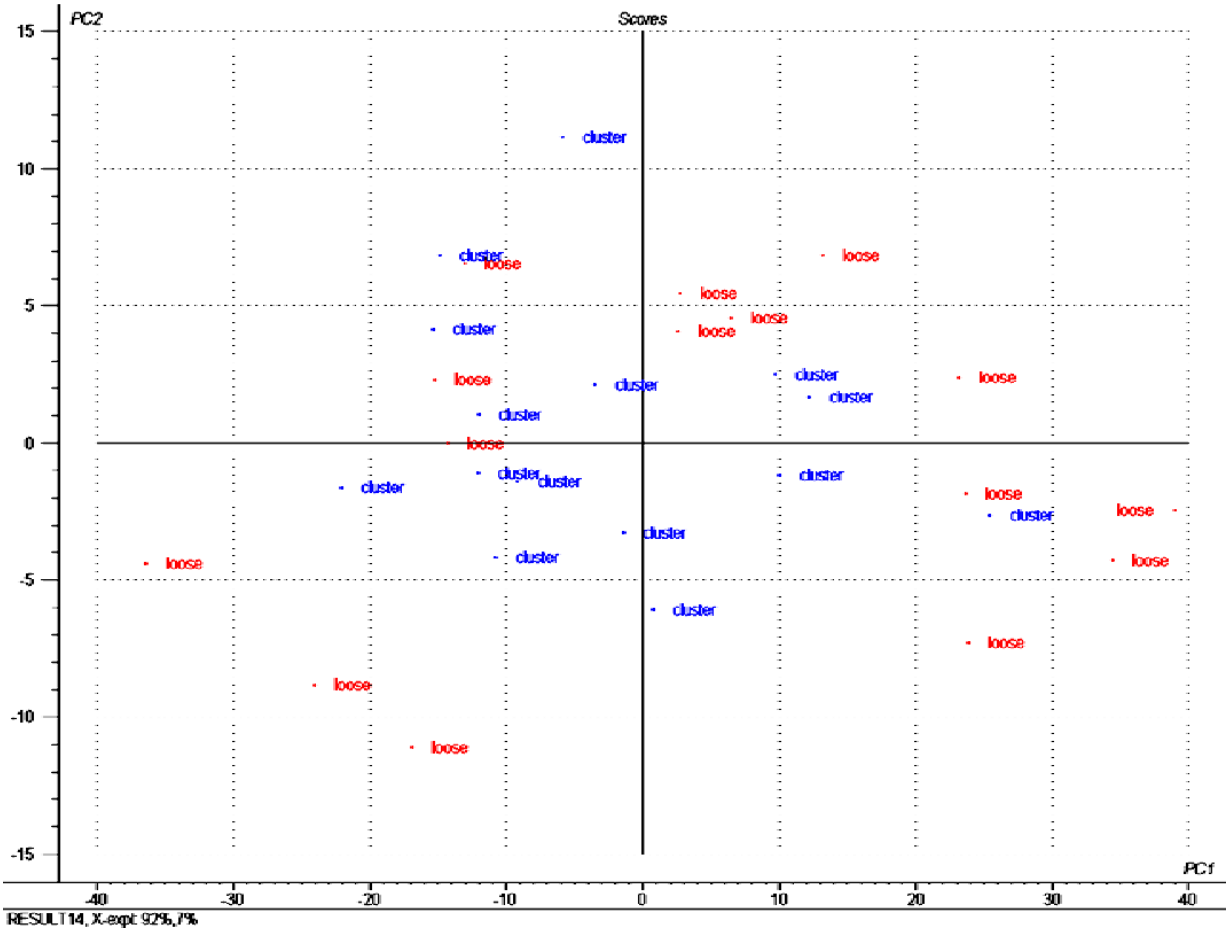


**Figure 4:** The scoreplot (PCA analysis) of the spectra of eight different tomato cultivars (loose and cluster) in the NIR spectral range (PC1 and PC2 explain 92% and 7% of the variability respectively).

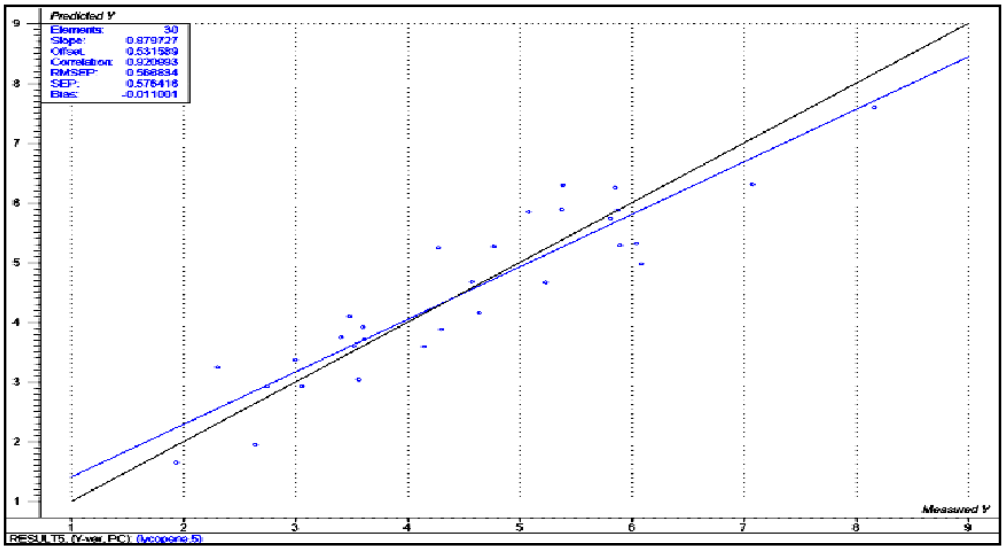


**Figure 5:** Calibration model (PLS) for lycopene content in eight tomato cultivars as measured in the visible spectral range.

components explained 80% and 12% of the total variance respectively.

The PLS prediction result for lycopene in the visible spectra is presented in Figure 5. In this figure, the ordinate and abscissa axes respectively represent the predicted and measured values of lycopene in tomato. A summary of the results that show the performance of the model in the different spectral ranges (Vis, NIR and full range) is also presented in Table 2.

The PLS model can predict the lycopene content of tomato in visible, Vis/NIR and NIR spectral ranges with a correlation coefficient (*r*) of 0.92, 0.90 and 0.82 respectively. A reasonable model was obtained by using the full spectral range (*r* = 0.90, RMSEP = 0.64, SEP = 0.65, *bias* = -0.012) and the NIR wavelength range (*r* = 0.82, RMSEP = 0.87, SEP = 0.88, *bias* = 0.028). However, the best model was obtained when the spectral range was limited to 380 – 950 nm (*r* = 0.92, RMSEP = 0.57, SEP = 0.58, bias = -0.011) for lycopene (Table 2 and Figure 5). The wave length range (380 - 950 nm) which is in the visible spectra seems more convenient for prediction of lycopene in tomato. It seems true that relatively higher spectral ranges are disturbed by $CO_2$ absorption as explained by Baranska *et al*. (2006). In general, the multivariate calibration model PLS had the potential to estimate the lycopene component concentrations in the different cultivars and the most estimate prediction was based on the visible spectra, which means the color of the tomatoes. Obviously measurement of color is closely related to visual perception (Shewfelt *et al*. 1988). Also the PCA analysis showed that the loose and cluster tomatoes could be separated from each other based on the visible spectra.

This can lead to the conclusion that Vis/NIR spectroscopy has allowed for rapid, simple and accurate determination of lycopene in tomatoes with minimal sample preparation. The result suggests the potential of Vis/NIR spectroscopy for determining the status of lycopene in a non-destructive way. From this study it can be generalized that this technique has very promising results but more measurements should be performed on a large range of tomato cultivars.

## CONCLUSION

The study has evaluated the non-destructive Vis/NIR reflectance spectroscopy measurement of lycopene in the same tomato cultivars at different spectral ranges. The Vis/NIR spectroscopy combined with multivariate calibration method PCA and PLS has the potential to estimate the concentration of lycopene in tomatoes. With this technique, a reasonable prediction performance was achieved from the established PLS model for lycopene at full spectral range (380–1700 nm), *r* =0.90 and SEP=0.65 mg/100g. However, the best model was obtained when the spectral range was limited to the visible range (380–950 nm), *r* = 0.92 and SEP=0.58 mg/100g. Therefore, it can lead to the conclusion that, although HPLC technique is a widely accepted method of choice, Vis/NIR spectroscopy can be used for rapid, simple and accurate determination of lycopene in tomatoes

with minimal sample preparation. However, more measurements should be performed on a large range of tomato cultivars and/or on other related fruits such as watermelon.

## REFERENCES


Ali, A., and Rao, A.V (2007). "Biologically active phytochemicals in human health: Lycopene." *International Journal of Food Properties* 10:279-288.

Ananthanarayan, L., and Choudhari, S.M (2007). Enzyme aided extraction of lycopene from tomato tissues. *Food Chemistry* 102: 77- 81.

Barba, A.I.O., Ca´mara Hurtado, M., Sa´nchez Mata, M.C., Ferna´ndez Ruiz, V. and Lo´pez Saénza de Tejada, M. (2006). Application of a UV - vis detection - HPLC method for a rapid determination of lycopene and β-carotene in vegetables. *Food Chemistry* 95: 328-336.

Baranska, M., Schulze, W. and Schulz, H. (2006). "Determination of lycopene and B-carotene content in tomato fruits and related products: Comparison of FT-Raman, and NIR spectroscopy." *Anals Chemistry* 78: 8456-8461.

Berna, A. (2006). Fast instrumental techniques to analyse the aroma of tomatoes. PhD Dissertationes de agricultura. Leuven: Katholieke Universitiet , Leuven.

Berna, A.Z., Saevels, S., Lammertyn, J. and Nicolai, B.M. (2002). Study of system effect on the aroma profile of intact tomatoes (Lycopersicum esculentum Mill.) by means of the electronic nose." Proceedings of the 9th International Symposium on Olfaction and Electronic Nose, ISOEN´02, Roma-Italy.

Di Mascio, P., Kaiser, S and Sies, H. (1989). Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Archives of Biochemistry and Biophysics* 274: 532.

Gomez-Romero, M., Arraez-Roman, J., Segura-Carretero, A and Fernandez-Gutierrez, A. (2007). Analytical determination of antioxidants in tomato: Typical components of the Mediterranean diet. *Journal of Seperation Science* 30: 452-461.

Maguer, M.L., and Shi, J. (2000). Lycopene in Tomatoes: Chemical and Physical Proporties Affected by Food Processing. *Critical Reviews in Food Science and Nutrition* 40(1):1-42.

Peirs, A., Desmet, M., Nicholai, B. and Buyssens, S. (2003). Relations between sensoy analysis, instrumental quality and NIR measurments of tomato qualiy. Edited by J.Oosterhhaven & H.W.Peppelenbos. *Proc.8th Int.CA Conference.* Acta Hort 600, ISHS, 471-477.

Pol, J., yotylainena, T., Ranta-Aho, O. and Riekkola, M.L. (2004). Determination of lycopene in food by on-line SFE coupled to HPLC using a single monolithic column for trapping and separation. *Journal of Chromatography A.* 1052: 25 - 31.

Qiu, W., Jiang, H., Wang, H. and Gao, Y. (2006). Effect of high hydrostatic pressure on lycopene stability. *Food Chemistry* 97:516-523.

Rao, L.G. and Rao, A.V. (2007). Carotenoids and human health. *Pharmacological Research* 55:207-216.

Rudnitskaya, A., Legin, A., Salles, C. and Mielle, P. (2001). Tomato varieties evaluation: electronic tongue vs. chemical analysis and sensory panel. Edited by Stetter, J.R and Penrose, W.R. Proceedings of the Artificial Chemical Sensing: Olfaction and the Electronic Nose. Sensor Division, The Electrochemical Society Inc. USA, 2001-2015.

Shewfelt, R. L., Thai, C.M and Davis, J.W. (1988). Prediction of changes in color of tomatoes during ripening at different constant temperatures. *Journal of Food Science* 53: 1433–1437.

Shi, J., Maguer, M.L., Bryan, M. and Kakuda, Y. (2003). Kinetics of lycopene degradation in tomato puree by heat and light irradiation. *Journal of Food Process Engineering* 25: 485-498.

Xu, F., Yuan, Q.P. and Dong, H.R. (2006). Determination of lycopene and β-carotene by high-performance liquid chromatography using sudan I as internal standard. *Journal of Chromatography B* 838: 44-49.

Yong, H., Zhang, Y., Pereira, A.G., Gomez, A.H. and Wang. J. (2005). Nondestructive determination of tomato fruit quality characteristic using Vis/NIR spectroscopy technique. *International Journal of Information Technology* 11(11): 97-106.

Singh, B. and Singh, S. (2003). Antimicrobial activity of Terpenoids from *Trichodesma amplexicaule* Roth. *Phytotherapy Research* 17(7): 814-816.

Singh, R., Singh, S., Kumar, S. and Arora, S. (2007). Free radical-scavenging activity of acetone extract/fractions of *Acacia auriculiformis* A. Cunn. *Food Chemistry* 103: 1403-1410.

Taleb-Contini, S.H., Salvador, M.J., Watanabe, E., Ito, I.Y. and de Oliveira, D.C.R. (2003). Antimicrobial activity of Flavonoids and steroids isolated from two *Chromolaena* species. *Brazilian Journal of Pharmaceutical Sciences* 39(4): 403-408.

Tilak, J.C., Adhikari, S. and Devasagayam, T.P.A. (2004). Antioxidant properties of *Plumbago zeylanica*, an Indian medicinal plant and its active ingredient, plumbagin. Redox Report 9(4): 220-227.

Yen, G., Duh, P. and Su, H. (2005). Antioxidant properties of lotus seed and its effect on DNA damage in human lymphocytes. *Food Chemistry* 89: 379-385.

Zhishen, J., Mengcheng, T. and Janming, W. (1999). The determination of flavonoids contents in mulberry & their scavenging effects on superoxide radicals. *Food Chemistry* 64: 555-559.