Pak. j. sci. ind. res. Ser. B: biol. sci. 2013 **56** (2) 90-97

Simultaneous Spectrophotometric Determination of Lycopene and Beta-Carotene Concentrations in Carotenoid Mixtures of the Extracts from Tomatoes, Papaya and Orange Juice

Misbaudeen Abdul-Hammed*, Isah Adewale Bello and Sunday Olusegun Oladoye
Department of Pure and Applied Chemistry, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

(received February 2, 2012; revised June 26, 2012; accepted June 27, 2012)

Abstract. A simple and inexpensive spectrophotometric equation model for the simultaneous determination of lycopene and β-carotene concentrations in a mixture of carotenoids is proposed. Lycopene could be exclusively determined (with the relative accuracy of more than 95%) using the absorbance data at 502 nm. Because quantifying the β-carotene concentration in a ccarotenoid minture using the sole absorbance at 450 nm is prone to error, an equation to determine the concentration of this compound from the absorbances data at two wavelengths was modeled. Using the modeled equations to re-check the molar absorptivity of lycopene at 472 nm, the value obtained was about 98% close to the value reported in literature. The relative accuracy of the predicted concentrations of two carotenoids using the modeled equations is a function of the ratio of these carotenoids in the samples.

Keywords: lycopene, β-carotene, spectrophotometry, absorptivities, tomato, papaya, orange, isoprene

Introduction

Carotenoids, the C_{40} tetraterpenoids derived from head-to-tail condensation of eight isoprenoid units, are notable for their wide distribution, structural diversity, and various functions. More than 600 carotenoids, excluding *cis* and *trans* isomers, have been isolated and characterized from natural sources (Pfander, 1987). Carotenoids have been credited with several beneficial effects on human health ranging from provitamin A activity to the enhancement of the immune response and reduction of the risk of degenerative diseases such as cancer, cardiovascular diseases, cataract, and macular degeneration (Olson 1999a; Astrog, 1997; Burri 1997; Mayne, 1996; Olson and Krinsky, 1995; Bendich, 1994; Krinsky, 1994; Gaziano and Hennekens, 1993). The action of carotenoids against chronic diseases has been mainly attributed to an antioxidant property, specifically, their ability to quench singlet oxygen and interact with free radicals (Palozza and Krinsky, 1992). However, other mechanisms, such as modulation of carcinogen metabolism, inhibition of cell proliferation, enhancement of cell differentiation and stimulation of cell-to-cell communication have been reported (Olson, 1999a; 1999b). The ability of carotenoids to quench singlet oxygen has been linked to the conjugated double bond system, the maximum efficiency being shown by carotenoids with nine or more conjugated double bonds (Foote *et al.*, 1970). Lycopene was found to be twice more efficient than the dicyclic β-carotene (Di Mascio *et al.,* 1989), despite of both compounds possessing 11conjugated double bonds. The effects of lycopene on human health as an antioxidant protector against lung, stomach, and prostate cancers have attracted considerable interest (Giovannucci, 1999; Clinton, 1998; Sies and Stahl, 1998; Gerster, 1997; Stahl and Sies, 1996). Average daily dietary lycopene intake levels (assessed by means of food frequency questionnaire) were estimated to be 25.2 and 33.39 mg/day in the Canadian and Nigerian diets, respectively (Olajire *et al*., 2007; Rao *et al*., 1998).

Carotenoid analysis is inherently difficult and error prone. In developed countries, separation and quantification of carotenoids are usually carried out using high-performance liquid chromatography (HPLC) technique (Monge-Rojas and Campos, 2011; Barba *et al.,* 2006). This method, although is reliable, accurate and time-consuming it requires extensive sample preparation, the use and disposal of hazardous organic solvents. It also requires highly trained personnel and expensive equipments. Novel chemical techniques that have been evaluated for direct determination of lycopene

*Author for correspondence; E-mail: misbaulhameed@ yahoo.co.uk

and other nutritionally important carotenoids include the application of optothermal and photothermal methods (Bicanic, 2011; Bicanic *et al*., 2005; 2004). These methods are gaining the popularity because of their simplicity. Other commonly used methods include resonance Raman spectroscopy (Bhosale *et al*., 2004), infrared (De Nardo *et al*., 2009; Halim *et al*., 2006) and near infrared spectroscopy (Baranska *et al*., 2006; Pedro and Ferreiram, 2005). Fourier transform infrared (FTIR) in combination with multivariate analysis offers a powerful and rapid technique for the analysis of agricultural and food products (Jha and Matsuoka, 2000). Conventional UV-visible spectrophotometric assays have also been shown to be simple, rapid, and inexpensive methods for measuring lycopene content in tomato and tomato products (Biswas *et al*., 2011; Davis *et al*., 2003), but absorbance interference from lycopene provides poor accuracy and overestimation of β-carotene levels (Olives-Barba *et al*., 2006). However, in some developing nations with limited accessibility to HPLC, the quantification of lycopene and β-carotene is often accomplished by means of the traditional ultraviolet-visible spectrophotometry. This method should be accurate enough, in that it has been shown that the content of lycopene in samples determined by the use of both experimental (calibration curve) and theoretical data (Beer-Lamberts law) shows a good agreement, with a relative error below 3% (Ravelo-Pérez *et al*., 2008). Some published absorption coefficients values may contain significant levels of error or uncertainty (Britton, 1995), likewise different authors choose different absorption coefficients for same carotenoids (in the same solvents), thus accounting for a good part of the variations in analytical results. In some studies the spectrophotometer detector was set at 472 nm to quantify lycopene (Moraru and Lee, 2005; Sharma and Le Maguer, 1996) and at 436 nm (Lumpkin, 2005) while in others it was determined at 503 nm (Ravelo-Perez *et al*., 2008). Although the absorbance of lycopene at 503 nm is not the highest, it was so selected to avoid interferences from other carotenoids present in the samples. The values of λ_{max} for carotenoids in hexane and petroleum ether are practically the same for diethyl ether, methanol, ethanol and acetonitrile, and 2-6 nm, 10-20 nm, 10-20 nm and 18-24 nm higher in acetone, chloroform, and *n* dichloromethane as well as in toluene, respectively (Britton, 1995). However, in a carotenoid mixture containing lycopene and β-carotene, the choice of absorption wavelengths of 450 nm and 470 nm to quantify β-carotene and lycopene, respectively may not reflect the true concentrations of each of the two carotenoids, since both absorb significantly at those two wavelength ranges, while the choice of 502 nm is perfect for lycopene determination. In the present work, the model equations for simultaneous determination of beta-carotene and lycopene concentrations in hexane layer extract of lycopene/β-carotene mixtures have been derived. This was then used to quantify β-carotene and lycopene in separate hexane extracts of the carotenoid mixtures from tomatoes, papaya and orange juice samples.

Materials and Methods

Tomato fruit samples (Ibadan-local cultivar bought from a farmland near Wazobia Market, Ogbomoso, Nigeria) were randomly selected and packed into nylon bags and quickly taken into the laboratory, then they were rinsed with distilled water and left to drain for some minutes. About 500 g (15-25 fruits) of fresh tomatoes was chopped, blended and then homogenized in a laboratory homogenizer. Conventional solvent extraction methods (Perkins-Veazie *et al.,* 2001; Sadler *et al.*, 1990) were employed for carotenoid extraction. Approximately 10g of the tomato serum was subjected to extraction with hexane, methanol and acetone (2:1:1) containing 2.5% butylated hydroxytoluene (BHT). The extract was treated with distilled water, methanol and 20 % KOH/methanol (1:1:1) to saponify any triglyceride present. The extract was then washed with distilled water and re-dissolved in hexane. The hexane extracts were scanned in the visible light wavelength range of 400-750 nm using HEλI0Sα UV-visible spectrophotometer (in a 1 cm path length quartz cuvette blanked with *n*-hexane) and the maximum absorbances were observed at 450, 472 and 502 nm, respectively for the lycopene - β-carotene hexane layer mixture. The mixture was diluted with *n*-hexane using dilution factors of 2, 3, 6, 8 and 16, respectively to check for result consistencies and subsequent absorbances were measured. Samples of papaya and orange juice extracted in hexane were also subjected to carotenoid analysis. The molar extinction coefficient of 172,000 L/mol/cm at 502 nm was used to estimate lycopene concentration, using the Beer-Lamberts law (Ravelo-Perez *et al*., 2008; Zechmeister and Polgar, 1943).

Results and Discussion

Figure 1A-B represent typical overlap spectra of the hexane extract of the lycopene/β-carotenoid mixture in tomatoes with absorption maxima at 450 nm, 472 nm

and 502 nm. Most carotenoids exhibit absorbance maxima at three wavelengths, resulting in a three-peak spectrum. As the number of conjugated double bonds increases, the λ_{max} shifts to longer wavelengths. Thus, the most unsaturated acyclic carotenoid, lycopene, with 11 conjugated double bonds is red and absorbs at the longest wavelengths (λ_{max} at 443, 471, 502 nm) (Rodriguez-Amaya and Kimura, 2004). Cyclization results in steric hindrance between the methyl group at C-5 of the ring and the hydrogen atom at C-8 of the polyene chain. This hindrance takes the π electrons of the ring double bond out of plane with respect to those of the chain, causing a hypsochromic shift (displacement of λ_{max} to shorter wavelength), a hypochromic effect (decrease in absorbance) and loss of fine structure (spectrum with less defined peaks). Thus, the dicyclic molecule, β-carotene, is yellow-orange and despite possessing the same number of conjugated double bonds as lycopene, exhibits absorption peaks at 450 and 472 nm and a mere shoulder at 425 nm (Rodriguez-Amaya and Kimura, 2004). This is so because both carotenoids absorb substantially in the overlapping wavelength ranges. As already stated that the choice of 502 nm to quantify lycopene is alright despite the fact that this wavelength value is not equal to λ_{max} (Ravelo-Perez *et al*., 2008). The correlations of the absorbances of the mixture at 450 nm and 502 nm relative to those at 472 nm, respectively (Fig. 2A-B) is very high ($R^2 = 0.999$ in both cases). This makes quantification of these carotenoids in mixture more ambiguous. Concentrations of lycopene and β-carotene in the mixture were calculated (Table 1) based on the assumption that the absorbances at 450 nm and at 502 nm are exclusively, for β-carotene and lycopene respectively. This is to be re-calculated using the proposed model and will then be compared henceforth. However, analytes such as

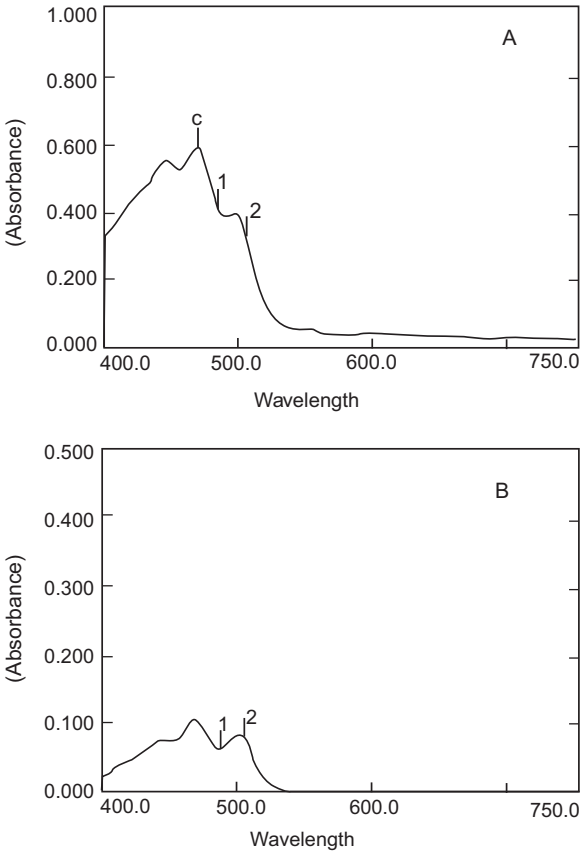

Fig. 1A-B. UV-visible spectra of *n*-hexane extract of the lycopene/β-carotene mixture of tomato samples at dilution factors of (A) 1 (B) 16.

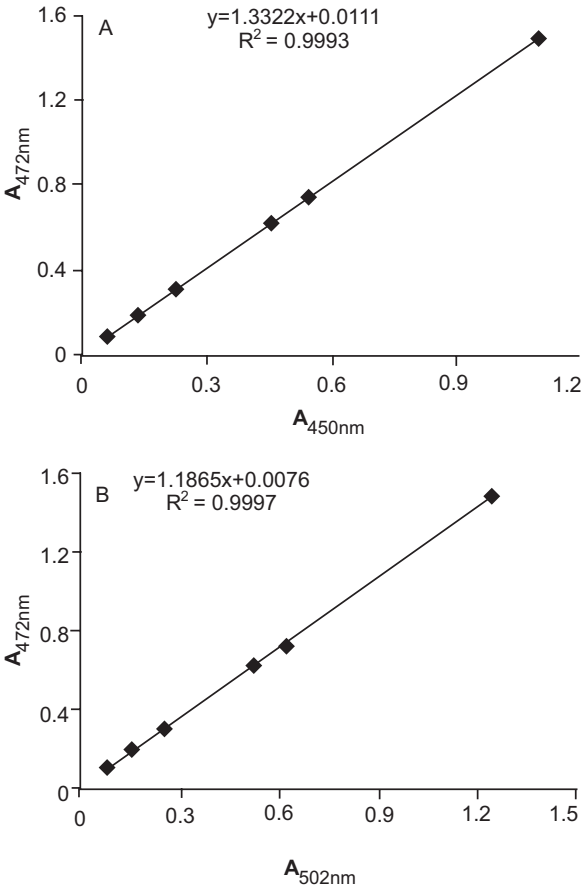

Fig. 2A-B. The correlations between the absorbances measured at 450nm (A) and 502 (B) nm to that measured at 472 nm for the hexane extract of the lycopene/β-carotene mixture of tomato samples.

Table 1. The ideal molar concentrations of lycopene and β-carotene in tomatoes, (Ibadan- local cultivars) in case that absorbances at 450nm and 502 nm are exclusively due to β-carotene and lycopene, respectively

Dilution factors	Absorbances (nm)			[a] β-Carotene concentration ± SD (μM)	[b] Lycopene concentration ± SD (μM)
	450 nm	472	502		
1	1.100	1.487	1.240	7.929 ± 0.202	7.209 ± 0.175
2	0.550	0.720	0.615	3.965 ± 0.156	3.576 ± 0.129
3	0.453	0.620	0.516	3.265 ± 0.162	3.000 ± 0.173
6	0.225	0.305	0.250	1.622 ± 0.224	1.453 ± 0.032
8	0.133	0.191	0.152	0.959 ± 0.102	0.884 ± 0.205
16	0.068	0.109	0.080	0.490 ± 0.126	0.465 ± 0.092

[a] = Calculated from Beer-Lamberts Law using the absorbances at 450 nm and molar absorptivity of 1.39×10^5 L mol/cm/ (Du *et al*., 1998); [b] = Calculated from Beer-Lamberts Law using the absorbances at 502nm and molar absorptivity of 1.72×10^5 L mol/cm (Markovic *et al*., 2006); The results are the means of triplicate analysis with reported standard deviation (SD).

norfloxacin, ofloxacin and lomerfloxacin in their mixture have been determined simultaneously using chemometric method (Huang *et al*., 2009) and other analytes such as chlorpromazine, perphenazine and acetopromazine were quantified by kinetic wavelength-pair method (Carreto *et al*., 1997). Here, lycopene and β-carotene concentrations are determined by partial least squares calibration (Skoog *et al*., 2000). This involves determination of the absorbances of several solutions at the wavelength at which analytes, lycopene and β-carotene, absorb. The wavelengths were carefully chosen such that the molar absorptivity of one component is much larger than that of the second component. Therefore, absorbances at 450 nm and 502 nm were used where the molar absorptivities of β-carotene are 1.39×10^5 and 2.63×10^4 L/mol/cm, respectively and those of lycopene being 1.16×10^5 and 1.72×10^5 L/mol/cm, respectively. According to the law of Lamberts and Beer, the absorbance at 450 nm and 502 nm (in a 1 cm path-length quartz cuvette) of the carotenoid mixture of lycopene and β-carotene can be expre as follows:

$$A_{450} = \varepsilon^{450}_{lycopene}[Lycopene] + \varepsilon^{450}_{\beta-carotene}[\beta-carotene] \quad \text{..... (1)}$$

$$A_{502} = \varepsilon^{502}_{lycopene}[Lycopene] + \varepsilon^{502}_{\beta-carotene}[\beta-carotene] \quad \text{..... (2)}$$

where, A_{450} and A_{502} are the absorbances (at 1 cm cell path-length) of the lycopene/ β-carotene mixture in the hexane extract at 450 nm and 502 nm, respectively; [lycopene] and [β-carotene] are molar concentrations of lycopene and β-carotene, resspectively; $\varepsilon^{450}_{\beta\text{-}carotene}$ and $\varepsilon^{450}_{lycopene}$ are the molar absorptivities of lycopene and β-carotene at 450 nm while $\varepsilon^{502}_{\beta\text{-}carotene}$ and $\varepsilon^{502}_{lycopene}$ represent the respective molar absorptivities of lycopene and β-carotene at 502 nm.

Solving equations (1) and (2) simultaneously, the molar concentration of lycopene from equation (1) could be expressed as:

$$[Lycopene] = \{A_{450} - \varepsilon^{450}_{\beta-carotene}[\beta\text{-}carotene]\} \Big/ \varepsilon^{450}_{lycopene} \quad \text{..... (3)}$$

Substituting equation (3) into equation (2), the concentration of β-carotene can be calculated as:

$$[\beta-carotene] = \frac{A_{450}\dfrac{\varepsilon^{502}_{lycopene}}{\varepsilon^{450}_{lycopene}} - A_{502}}{\dfrac{\varepsilon^{502}_{lycopene}}{\varepsilon^{450}_{lycopene}}\varepsilon^{450}_{\beta-carotene} - \varepsilon^{502}_{\beta-carotene}} \quad \text{..... (4)}$$

But the values $\varepsilon^{450}_{\beta\text{-}carotene}$, $\varepsilon^{450}_{lycopene}$, $\varepsilon^{502}_{\beta\text{-}carotene}$ and $\varepsilon^{502}_{lycopene}$ are known to be 1.39×10^5, 1.16×10^5, 2.63×10^4 and 1.72×10^5 L/mol/cm, respectively (Clinton, 1998; Du *et al.,* 1998; Krinsky *et al.,* 1990; Zechmeister and Polgar, 1943), therefore:

$$[\beta\text{-}carotene] = \frac{1.483\,A_{450} - A_{502}}{1.798\times10^5} \quad \text{..... (5)}$$

$$[Lycopene] = \frac{A_{450} - 1.39\times10^5[\beta\text{-}carotene]}{1.16\times10^5} \approx \frac{A_{502}}{1.72\times10^5} \quad \text{... (6)}$$

Equations (5) and (6) were employed to calculate the concentrations of lycopene and β-carotene, respectively

and the results are as presented in Table 2. It was clearly shown that the use of the absorbances at 450 nm to exclusively determine β-carotene concentration is extremely prone to error. This is so because the relative accuracy of the predicted β-carotene concentration using the modeled equations compared with what could have been reported when its concentration was calculated from their absorbance values at 450 nm, is extremely low (an average of 26.78%). However, it could be inferred that absorbance at 502 nm could be exclusively attributed to lycopene, since an average relative accuracy of more than 95% was obtained. This is in agreement with other study (Fish *et al*., 2002) which suggests that constituent carotenoids other than lycopene will contribute to the absorbance at 503 nm up to about 4% for fresh red tomatoes, 2% for red-fleshed watermelon and 6% for pink grapefruit. This also corroborated the findings that the lycopene contents in different varieties of tomatoes, analyzed by UV-visible spectrophotometry at 502 nm and HPLC methods, are quite similar (Laleye *et al*., 2010).

To check for the correctness of the lycopene concentration at 472 nm, equation 1 may be re-written as:

$$A_{472} = \varepsilon^{472}_{lycopene}[Lycopene] + \varepsilon^{472}_{\beta\text{-}carotene}[\beta\text{–}carotene] \quad \text{..... (7)}$$

Assuming $\varepsilon^{472}_{lycopene}$ to be unknown, it can be calculated thus:

$$\varepsilon^{472}_{lycopene} = \frac{A_{472} - \varepsilon^{472}_{\beta\text{-}carotene}[\beta\text{–}carotene]}{[Lycopene]} \quad \text{..... (8)}$$

Substituting the value of A_{472} and $\varepsilon^{472}_{\beta\text{-}carotene} = 1.09 \times 10^5$ L/mol/cm (Du *et al.,* 1998) as well as the concentrations of lycopene and β-carotene of the stock solution (solution of dilution factor of 1, that is 6.876 and 2.175 μM $\varepsilon^{472}_{\beta\text{-}lycopene}$ value of 1.82 x105 L/mol/cm was obtained as against a value of 1.86 x 105 L/mol/cm previously reported by Clinton (1998). This gives a relative accuracy (ratio of predicted value to the true value) of about 98%.

The absorption spectra of the hexane extracts samples of papaya and orange juice Fig. 3A-B are shown in Equations (5) and (6) were then tested on the absorbance

Table 2. Predicted concentrations of lycopene and β-carotene in the hexane extract from tomatoes (Ibadan-local cultivar)

Dilution factors	Absorbances (nm)			[a] β-Carotene concentration ± SD (μM)	[b] Lycopene concentration ± SD (μM)	[c] Relative accuracy	
	450	472	502			β-carotene at 450 nm	Lycopene at 502 nm
1	1.100	1.487	1.240	2.175 ± 0.127	6.876 ± 0.212	27.431	95.377
2	0.550	0.720	0.615	1.116 ± 0.062	3.404 ± 0.156	28.150	95.201
3	0.453	0.620	0.516	0.870 ± 0.152	2.866 ± 0.124	26.644	95.533
6	0.225	0.305	0.250	0.466 ± 0.094	1.381 ± 0.136	28.733	95.013
8	0.133	0.191	0.152	0.250 ± 0.022	0.847 ± 0.087	26.057	95.845
16	0.068	0.109	0.080	0.116 ± 0.013	0.448 ± 0.056	23.666	96.320
Average percentages	-	-	-	-	-	26.780	95.548

[a] = Calculated using equation (5); [b] = Calculated using equation (6); The results are the means of triplicate analysis with reported standard deviation (SD); [c] = Relative accuracy means the ratio of the predicted concentrations of lycopene and β-carotene when the modeled equations were used relative to what could have been reported when their concentrations were calculated from their absorbance values at 502 nm and 450 nm, the wavelengths assumed to be attributed exclusively to the respective carotenoids in their mixtures.

Table 3. Predicted concentrations of lycopene and β-carotene in papaya and orange juice

[a] Sample	Absorbances (nm)		[b] β-Carotene concentration (μM)	[c] Lycopene concentration (μM)	β-Carotene concentration (μg/g)	Lycopene concentration (μg/g)
	450	502				
Papaya	0.585	0.401	2.590 ± 0.132	1.934 ± 0.124	27.765 ± 1.415	20.732 ±1.329
Orange juice	0.123	0.051	0.731 ± 0.092	0.185 ± 0.022	7.836 ± 0.986	1.983 ±0.236

[a] = Sample weight is 10 g and the volume of hexane extract is 200 mL; [b] = calculated using equation (5); [c] = calculated using equation (6); The results are the means of triplicate analysis with reported standard deviation (SD).

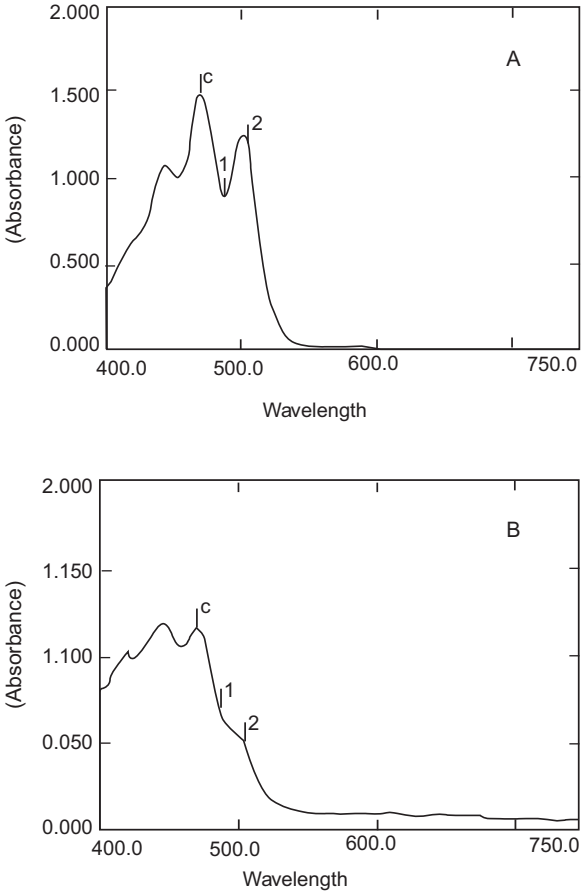

Fig. 3A-B. UV-visible spectra of *n*-hexane extract of the lycopene/β-carotene mixture in samples of (A) orange juice (B) papaya.

results of the hexane extract of papaya and orange juice samples to calculate the respective amounts of β-carotene and lycopene in the lycopene/β-carotene mixture. The results are presented in Table 3. The results with other fruit or fruit product apart from tomatoes confirm that the β-carotene concentrations are higher than that of lycopene. This is evident from the spectra shown in Fig. 3A-B. High relative accuracy values of the predicted β-carotene concentration relative to that computed exclusively at 450 nm of 61.42% and 82.45% were observed for papaya and orange juice, respectively in contrast with an average of 26.78% observed for tomato fruits. Furthermore, the relative accuracies (82.95% and 62.39%) of the predicted lycopene relative to that computed exclusively at 502 nm observed for papaya and orange juice, respectively are lower than an average of 95.55% obtained for tomatoes. This may be attributed to lycopene/ β-carotene concentration ratio in the sample under investigation or/and the presence of appreciable amounts of other hydrocarbon carotenoids in papaya and orange. It is hereby recommended that the predicted equations in this work be employed in the determination of concentrations of lycopene and β-carotene in the hexane extract of lycopene/ β-carotene mixture and the results can be compared with those from HPLC analysis, to check for precision and accuracy.

Conclusion

A simple and inexpensive spectrophotometric equation model for the simultaneous determination of lycopene and β-carotene concentrations in a carotenoid mixture is proposed. This could be useful to analysis in some developing nations where UV-visible spectrophotometry is available but accessibility to HPLC is minimal. The equations proposed here worked best if the percentage composition of other hydrocarbon carotenoids (relative to lycopene and β-carotene) in the hexane extract is minimal.

Acknowledgement

The authors acknowledge the support of German Academic Exchange Service (DAAD), Germany for the grant for the purchase of small equipments and materials offered to the corresponding author. The research aid to young researchers by the Faculty of Pure and Applied Sciences, LAUTECH, Ogbomoso is also acknowledged.

References

Astrog, P. 1997. Food carotenoids and cancer prevention: An overview of current research. *Trends in Food Science and Technology*, **81:** 406-413.

Baranska, M., Schutze, W., Schulz, H. 2006. Determination of lycopene and β-carotene content in tomato fruit and related products: Comparison of FT-Raman, ATR-IR, and NIR spectroscopy. *Analytical Chemistry*, **78:** 8456-8461.

Barba, A.I.O., Hurtado, M.C., Mata, M.C.S., Ruiz, V.F., de Tejada, M.L.S. 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.

Bendich, A. 1994. Recent advances in clinical research involving carotenoids. *Pure and Applied Chemistry*, **66:** 1017-1024.

Bhosale, P., Ermakov, I.V., Ermakova, M.R., Gellermann, W., Bernstein, P.S. 2004. Resonance Raman quantification of nutritionally important carotenoids in fruits, vegetables, and their juices

in comparison to high-pressure liquid chromatography analysis. *Journal of Agricultural and Food Chemistry*, **52:** 3281-3285.

Bicanic, D.D. 2011. On the photoacoustic, photothermal and colorimetric quantification of carotenoids and other phytonutrients in some foods: a review. *Journal of Molecular Structure*, **993:** 9-14.

Bicanic, D., Fogliano, V., Luterotti, S., Swarts, J., Piani, G., Graziani, G. 2005. Quantification of lycopene in tomato products: comparing the performances of newly proposed direct photothermal methods and high performance liquid chromatography. *Journal of the Science of Food and Agriculture*, **85:** 1149-1153.

Bicanic, D., Swarts, J., Luterotti, S., Pietraperzia, G., Dóka, O., de Rooij, H. 2004. 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. *Analytical Chemistry*, **76:** 5203-5207.

Biswas, A.K., Sahoo, J., Chatli, M.K. 2011. A simple UV-Vis spectrophotometric method for determination of β-carotene content in raw carrot, sweet potato and supplemented chicken meat nuggets. *Food Science and Technology*, **44:** 1809-1813.

Britton, G. 1995. UV/visible spectroscopy. In: *Carotenoids: Spectroscopy*, G., Britton, Liaaen- S., Jensen, and H. Pfander (eds), vol. **1B,** pp. 13-63, Birkhäuser Verlag, AG, Basel, Switzerland.

Burri, B.J. 1997. Beta-carotene and human health: A review of current research. *Nutrition Research*, **17:** 547-580.

Carreto, M.L., Lunar, L., Rubio, S., Perez-Bendito, D. 1997. Simultaneous spectrophotometric determination of chlorpromazine, perphenazine and acetopromazine by use of kinetic wavelength pair method. *Analytica Chimica Acta*, **349:** 33-42.

Clinton, S.K. 1998. Lycopene: Chemistry, biology, and implications for human health and disease. *Nutrition Reviews*, **56:** 35-51.

Davis, A.R., Fish, W.W., Perkins-Veazie, P. 2003. A rapid spectrophotometric method for analyzing lycopene content in tomato and tomato products. *Postharvest Biological Technology*, **28:** 425-430.

De Nardo, T., Shiroma-Kian, C., Halim, Y., Francis, D., Rodriguez-Saona, L.E. 2009. Rapid and simultaneous determination of lycopene and β-carotene contents in tomato juice by infrared spectroscopy. *Journal of Agricultural and Food Chemistry*, **57:** 1105-1112.

Di Mascio, P., Kaiser, S., Sies, H. 1989. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Archives of Biochemistry and Biophysics*, **274:** 532-538.

Du, H., Fuh, R.A., Li, J., Corkan, A., Lindsey, J.S. 1998. PhotochemCAD: A computer-aided design and research tool in photochemistry. *Photochemistry and Photobiology*, **68:** 141-142.

Fish, W.W., Perkins-Veazie, P., Collins, J.K.. 2002. A quantitative assay for lycopene that utilizes reduced volumes of organic solvents. *Journal of Food Composition and Analysis*, **15:** 309-317.

Foote, C.S., Chang, Y.C., Denny, R.W. 1970. Chemistry of singlet oxygen: Carotenoid quenching parallels biological protection. *Journal of the American Chemical Society*, **92:** 5216-5218.

Gaziano, J.M., Hennekens, C.H. 1993. The role of beta-carotene in the prevention of cardiovascular disease. *Annals of the New York Academy of Science*, **691:** 148-155.

Gerster, H.Y. 1997. The potential role of lycopene for human health. *Journal of the American College of Nutrition*, **16:** 109-126.

Giovannucci, E. 1999. Tomatoes, tomato-based products, lycopene, and cancer: Review of the epidemiologic literature. *Journal of National Cancer Institute*, **91:** 317-331.

Halim, Y., Schwartz, S.J., Francis, D., Baldauf, N.A., Rodriguez-Saona, L.E. 2006. Direct determination of lycopene content in tomatoes (*Lycopersion esculentum*) by attenuated total reflectance infrared spectroscopy and multivariate analysis. *Journal of Association of Official Analytical Chemist International*, **89:** 1257-1262.

Huang, X.G., Zhang, H.S., Li, Y.X., Li, M.F. 2009. Simultaneous spectrophotometric determination of Norfloxacin, Ofloxacin and Lomerfloxacin in rabbit blood serum by the use of chemometrics. *Journal of Chilean Chemical Society*, **54:** 204-207.

Jha, S.N., Matsuoka, T. 2000. Nondestructive techniques for quality evaluation of intact fruits and vegetables. *Food Science and Technology Research*, **6:** 248-251.

Krinsky, N.I. 1994. Actions of carotenoids in biological systems. *Annual Reviews of Nutrition*, **13:** 561-587.

Krinsky, N.I., Russett, M.D. Handelman, G.J., Snodderly, D.M. 1990. Structural and geometrical isomers of carotenoids in human plasma. *Journal of Nutrition*, **120:** 1654-1662.

Laleye, L.C., Al Hammadi, S.I., Jobe, B., Rao, M.V.

2010. Assessment of lycopene contents of fresh tomatoes (*Lycopersicon esculentum* Mill.) and tomato products in the United Arab Emirates. *Journal of Food, Agriculture and Environment*, **8:** 142-147.

Lumpkin, H. 2005. A comparison of lycopene and other phytochemicals in tomatoes grown under conventional and organic management systems. *Technical Bulletin No.* **34**, Asian Vegetable Research and Development Center (publication number 05-623), 48 pp., The World Vegetable Center, Shanhua, Taiwan.

Markovic, K., Hruskar, M., Vahcic, N. 2006. Lycopene content of tomato products and their contribution to the lycopene intake of croatian. *Nutritional Research*, **26:** 556-560.

Mayne, S.T. 1996. Beta-carotene, carotenoids, and disease prevention in humans. *Federation of American Societies for Experimental Biology Journal*, **10:** 690-701.

Monge-Rojas, R., Campos. H. 2011. Tocopherol and carotenoid content of foods commonly consumed in Costa Rica. *Journal of Food Composition and Analysis*, **24:** 202-216.

Moraru, C., Lee, T. 2005. Kinetic studies of lycopene isomerization in a tributyrin model system at gastric pH. *Journal of Agricultural and Food Chemistry*, **53:** 8997-9004.

Olajire, A.A., Ibrahim, A.O., Adelowo-Imeokparia F.E., Abdul-Hammed, M. 2007. Lycopene in tomato and tomato-based products: Levels and their contribution to dietary lycopene. *Pakistan Journal of Scientific Industrial Research*, **50:** 18-21.

Olives Barba, A.I., Camara Hurtado, M., Sanchez Mata, M.C., Fernandez Ruiz, V., Lopez Saenz 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.

Olson, J.A. 1999a. Carotenoids. In: *Modern Nutrition in Health and Disease*,M.E., Shils, J.A., Olson, M., Shike and A.C. Ross, (eds), pp. 525-541, 9th edition. Williams and Wilkins, Baltimore, USA.

Olson, J.A. 1999b. Carotenoids and human health. *Archivos of Latinoamericanos de Nutrition*, **49:** 7S-11S.

Olson, J.A., Krinsky, N.I. 1995. Introduction: The colorful, fascinating world of the carotenoids: Important physiologic modulators. *Journal of Federation of American Societies for Experimental Biology*, **9:** 1547-1550.

Palozza, P., Krinsky, N.I. 1992. Antioxidant effects of carotenoids *in vivo* and *in vitro*: An overview. *Methods in Enzymology*, **213:** 403-420.

Pedro, A.M.K., Ferreira, M.M.C. 2005. Nondestructive determination of solids and carotenoids in tomato products by near-infrared spectroscopy and multivariate calibration. *Analytical Chemistry*, **77:** 2505-2511.

Perkins-Veazie, P., Collins, J. K., Pair, S. D., Roberts, W. 2001. Lycopene content differs among red-fleshed watermelon cultivars. *Journal of the Science of Food and Agriculture,* **81:** 983-987.

Pfander, H. 1987. *Key to carotenoids,* 45 pp., 2nd edition, Birkhäuser Verlag, Basel, Switzerland.

Rao, A.V., Zeeshan, W., Sanjiv, A. 1998. Lycopene content of tomatoes and tomato products and their contribution to dietary lycopene. *Food Research International*, **31:** 737-741.

Ravelo-Pérez, L.M., Hernández-Borges, J., Rodríguez-Delgado, M.A., Borges-Miquel, T. 2008. Spectrophotometric Analysis of Lycopene in Tomatoes and Watermelons: A Practical Class. *Chemical Educator*, **13:** 11-13.

Rodriguez-Amaya, D.B., Kimura, M. 2004. *HarvestPlus Handbook for Carotenoid Analysis,* 63 pp., HarvestPlus Technical Monograph Series. **2,** Washington, DC. USA.

Sadler, G., Davis, J., Dezman, D. 1990. Rapid extraction of lycopene and β-carotene from reconstituted tomato paste and pink grapefruit homogenates. *Journal of Food Science*, **55:** 1460-1461.

Sharma, S.K., Le Maguer, M. 1996. Kinetics of lycopene degradation in tomato pulp solids under different processing and storage conditions. *Food Research International*, **29:** 309-315.

Sies, H., Stahl, W. 1998. Lycopene: Antioxidant and biological effects and its bioavailability in the human. In: *Proceedings of the Society of Experimental Biology and Medicine (New York, USA)*, **218:** 121-124.

Skoog, D.A., West, D.M., Holler, F.J., Crouch, S.R. 2000. *Analytical Chemistry: An Introduction,* pp. 604-608, 7th edition. Saunder College Publication, USA.

Stahl, W., Sies, H. 1996. Lycopene: A biologically important carotenoid for humans? *Archives of Biochemistry and Biophysics*, **336:** 1-9.

Zechmeister, L., Polgar, A. 1943. Cis-trans isomerization and spectral characteristics of carotenoids and some related compounds. *Journal of the American Chemical Society*, **65:** 1522-1528.