SIMULTANEOUS SPECTROPHOTOMETRIC DETERMINATION OF LYCOPENE AND BETA-CAROTENE CONCENTRATIONS IN CAROTENOID MIXTURES OF THE EXTRACT OF TOMATOES, *PAPAYA* AND ORANGE JUICE

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ABSTRACT

A simple and inexpensive spectrophotometric model for the simultaneous determination of lycopene and beta-carotene concentrations in a carotenoid mixture is proposed. Lycopene could be exclusively determined using the absorbance at 502nm with a relative accuracy of more than 95%. Determining the beta-carotene concentration at 450nm exclusively is extremely prone to error, but an equation for its determination from the absorbances at two wavelengths was modeled. A relative accuracy of about 98% was obtained when the use of the model equation was employed at a wavelength of 472nm. However, percentage relative absorbance coefficient of β-carotene relative to that of lycopene and vice versa, at the respective wavelengths is a function of the ratio of the carotenes present in the samples.

Key words: Lycopene, beta-carotene, spectrophotometry, absorptivities, tomato, 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 (Gaziano and Hennekens, 1993; Krinsky, 1993; Bendich, 1994; Olson and Krinsky, 1995; Mayne, 1996; Astrog, 1997; Burri 1997; Olson 1999a). The action of carotenoids against diseases has been 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 1999 a,b). 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 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 (Clinton, 1998; Gerster, 1997; Giovannucci, 1999; Sies and Stahl, 1998; 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 (Rao *et al*., 1998; Olajire *et al*., 2007).

Carotenoid analysis is inherently difficult and error prone. Despite substantial improvements and refinements in the methods for analyzing these fascinating but complicated compounds, discrepancies in analytical data can still be perceived in the literature. Food samples typically

contain both the non-polar carotenes and the more polar xanthophylls. The xanthophylls are removed through saponification process while only the hydrocarbon carotenes, predominantly lycopene and β-carotene, are retained in hexane layer. These are usually separated further using high performance liquid chromatography, HPLC. However, in some developing nations where accessibility to HPLC is minimal, it is projected that the concentrations of lycopene and β-carotene can be determined simultaneously using only Ultraviolet-Visible spectrophotometry. This method should be accurate enough, in that it has been shown that the lycopene content of samples determined by the use of both experimental (calibration curve) and theoretical data (Beer-Lamberts law) shows good agreement, with a relative error below 3% (Ravelo-Pérez *et al*., 2008). Therefore the instability of lycopene and carotene standards can be overcomed with the use of Beer-Lamberts law once their molar absorptivities at desired wavelengths are known. Some published absorption coefficients values may contain significant levels of error or uncertainty (Britton, 1995), and different authors choose different absorption coefficients for some carotenoids (in the same solvents), thus accounting for a good part of the variations in analytical results. In a study to measure lycopene concentration, the spectrophotometer detector was set at 472 nm (Sharma and Le Maguer, 1996; Moraru, and Lee, 2005), at 436 nm (Lumpkin, 2005) while in others, it was determined at 503 nm (Ravelo-Perez *et al*., 2008). The absorbance at 503 nm (A_{503}) was selected to avoid interferences from other carotenoids present in the samples, although the absorbance at this wavelength value is not the highest absorbance of lycopene in n-hexane (Ravelo-Perez *et al*., 2008). The λ_{max} values of carotenoids in hexane or petroleum ether are practically the same as in diethyl ether, methanol, ethanol, and acetonitrile, and higher by 2–6 nm in acetone, 10-20 nm in chloroform, 10-20 nm in dichloromethane and 18-24 nm in toluene (Britton, 1995). However, the choice of absorption wavelengths of about 440-

450 nm as well as about 470 nm is not the best for determining lycopene and beta-carotene concentrations solely in a carotenoid mixture, since both carotenoids absorb at the wavelength ranges, but the choice of 502nm is perfect for lycopene determination.

The present work is designed to propose model equations for the simultaneous determination of the concentration of beta-carotene and lycopene in the hexane layer extract lycopene/β-carotene mixtures of tomato samples at 450 nm, a wavelength at which both carotenoids absorb, using Beer-Lamberts law.

METHODS

Tomato fruit samples (bought at *Wazobia* Market, Ogbomoso, Nigeria) were randomly selected and packed into nylon bags and taken into the laboratory, where they were rinsed with some distilled water and left to drain for some minutes. Individual tomatoes were sliced and all parts of the fruits were utilized. The fruit tissue was cut into approximately 1.4 - 2.6 cm cubes. About 25-500 g of fresh tomato samples was homogenized. Conventional solvent extraction method (Sadler *et al.*, 1990) was employed for carotenoid extraction. Lycopene and β-carotene from the tomato fruits were extracted 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 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 hexane extract were also subjected to carotenoid

analysis. The molar extinction coefficient of 172,000 L $mol^{-1}cm^{-1}$ at 502nm was used to estimate lycopene concentration, using Beer-Lamberts law (Zechmeister and Polgar, 1943, Ravelo-Perez *et al*., 2008).

RESULTS AND DISCUSSIONS

Figures 1A and 1B represent typical overlap spectra of the hexane extract of the lycopene/ β-carotene mixture in tomato extract with absorption maxima at 450nm, 472 nm and 502nm. Most carotenoids absorb maximally 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). 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 exhibits absorption peaks at 450 and 472 nm and a mere shoulder at 425 nm, despite possessing the same number of conjugated double bonds as lycopene (Rodriguez-Amaya and Kimura, 2004). However, measuring the absorbances solely at absorption wavelengths of about 450 nm and 470 nm is not the best choice of determining lycopene and β-carotene concentrations, since both carotenoids absorb at the overlapping wavelength ranges simultaneously and significantly, but the choice of 502nm seems perfect for lycopene concentration determination, although the absorbance at this wavelength value is not the highest absorbance of lycopene in n-hexane (Ravelo-Perez et al., 2008). The correlations of the absorbances of the mixture at 450nm and

502 nm relative to those at 472nm (Figures 2A and 2B respectively) is very high (R^2 = 0.999 in both cases). This further makes the determination of the concentrations of these carotenoids in their mixture more ambiguous. The concentrations of lycopene and β-carotene in the mixture were calculated (Table 1) based on the assumption that the absorbances at 450nm and at 502nm 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 norfloxacin, ofloxacin and lomerfloxacin in their mixture have been determined simultaneously using chemometric method (Huang *et al*., 2009) and others by kinetic wavelength-pair method (Carreto *et al*., 1997). Here, the lycopene and β-carotene concentrations are determined by partial least squares calibration (Skoog *et al*., 2000). This involves the determination of the absorbances of several solutions at the wavelength at which analytes, lycopene and β-carotene, absorb. The careful choices of the wavelengths were made, such that the molar absorptivity of one component will be much larger than that of the second component. Therefore, absorbances at 450nm and 502 nm were used where the molar absorptivities of β-carotene are 1.39 x $10^5$and 2.63 x 10^4 $Lmol^{-1}cm^{-1}$ respectively, and those of lycopene being 1.16 x 10^5 and 1.72 x 10^5 $Lmol^{-1}cm^{-1}$ respectively. The absorbances of the carotenoid mixture of lycopene and β-carotene at 450nm and 502nm, using Beer-Lamberts law, can be expressed as follows:

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

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

where $A_{450}\,and\,A_{502}$ are absorbances of the lycopene/ β-carotene mixture hexane extract at 450 nm and 502nm respectively; [lycopene] and [β-carotene] are molar concentrations of lycopene and β-carotene respectively; $\varepsilon^{450}_{\beta-carotene}$ and $\varepsilon^{450}_{lycopene}$ are the molar absorptivities of lycopene and β-

carotene at 450nm while $\varepsilon^{502}_{\beta-carotene}$ and $\varepsilon^{502}_{lycopene}$ represent the respective molar absorptivities of lycopene and β-carotene at 502nm.

Solving equations (1) and (2) simultaneously,

The molar concentration of lycopene from equation (1) could be expressed as:

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

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

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

But the values $\varepsilon^{450}_{\beta-carotene}$, $\varepsilon^{450}_{lycopene}$, $\varepsilon^{502}_{\beta-carotene}$ and $\varepsilon^{502}_{lycopene}$ are known to be 1.39 x 10^5, 1.16 x 10^5, 2.63 x 10^4 and 1.72 x 10^5 L $mol^{-1}cm^{-1}$ respectively (Zechmeister and Polgar, 1943; Krinsky, *et al.*, 1990; Du *et al*., 1998; Clinton, 1998), therefore:

$$[\beta-carotene] = \frac{1.483A_{450} - A_{502}}{1.798x10^5} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

$$[Lycopene] = \frac{A_{450} - 1.39x10^5[\beta-carotene]}{1.16x10^5} \approx \frac{A_{502}}{1.72x10^5} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (6)$$

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 450nm to exclusively determine β-carotene concentration is extremely prone to

error, since % relative absorbance coefficient of β-carotene relative to that of lycopene at 450nm is extremely low (an average of 26.78%). However, it could be inferred that absorbance at 502nm could be exclusively attributed to lycopene, since an average % relative absorbance coefficient of more than 95% was obtained for lycopene relative to that of β-carotene. This corroborated the findings that the lycopene contents in different varieties of tomatoes, analyzed by UV-visible spectrophotometry and HPLC methods, are quite similar (Laleye *et al*., 2010).

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

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

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

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

Substituting the values of A_{472} and $\varepsilon^{472}_{\beta-carotene}$ = 1.09 x 10^5 L $mol^{-1}cm^{-1}$ (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), we obtained $\varepsilon^{472}_{lycopene}$ value of 1.82 x 10^5 L $mol^{-1}cm^{-1}$ as against a value of 1.86 x 10^5 L $mol^{-1}cm^{-1}$ previously reported (Clinton, 1998). This gives a relative accuracy of about 98%.

The absorption spectra of the hexane extracts of samples of papaya and orange juice are shown in Figures 3A and 3B. Equations (5) and (6) were then tested on the absorbance results of the hexane extract of samples of papaya and orange juice to calculate the respective amounts of β-carotene and lycopene in the lycopene/ β-carotene mixture. The results are as shown in Table 3. The results with other fruit or fruit product apart from tomatoes show that the β-carotene

concentrations are higher than that of lycopene. This is evident from the spectra shown in Figures 3A and 3B. High % relative absorbance coefficient values of β-carotene relative to that of lycopene at 450nm of 61.42% and 82.45% for papaya and orange juice respectively were observed, in contrast with an average of 26.78% observed for tomato fruits. Also, lower % relative absorbance coefficient values of lycopene relative to that of β-carotene at 502nm were observed, 82.95% and 62.39% for papaya and orange juice respectively, as against an average of 95.55% for tomatoes. This may be attributed to lycopene/ β-carotene concentration ratio in the sample under investigation. 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 be compared with those from HPLC analysis, to check for precision and accuracy.

CONCLUSIONS

A simple and inexpensive spectrophotometric model for the simultaneous determination of lycopene and beta-carotene concentrations in a carotenoid mixture is proposed. This could be useful to analysts 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 carotene (relative to lycopene and β-carotene) in the hexane extract is very minimal.

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Table 1: The ideal molar concentrations of lycopene and β-carotene (if the absorbances at 450nm and 502 nm are exclusively for β-carotene and lycopene respectively)

Dilution Factors	Absorbances			[a] β-Carotene concentration ± SD (μM)	[b] Lycopene Concentration ± SD (μM)
	At 450nm	At 472nm	At 502nm		
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 450nm and molar absorptivity of 1.39×10^5 L $mol^{-1}cm^{-1}$ (Du *et al*., 1998).

[b] Calculated from Beer-Lamberts law using the absorbances at 502nm and molar absorptivity of 1.72×10^5 L $mol^{-1}cm^{-1}$ (Markovic *et al*., 2006).

The results are the means of triplicate analysis with reported standard deviation (SD).

Table 2: The actual molar concentrations of lycopene and β-carotene (calculated when β-carotene and lycopene are treated as independent species in a mixture with overlapping absorbances)

Dilution Factors	Absorbances			[a] β-Carotene concentration ± SD (μM)	[b] Lycopene concentration ± SD (μM)	[c] % RAC	
	At 450nm	At 472nm	At 502nm			β-carotene at 450nm	Lycopene at 502nm
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] % RAC = % Relative Absorbance Coefficient indicating the fraction of absorbance values of β-carotene relative to that of lycopene at 450nm and fraction of absorbance values of relative to that of β-carotene at 450nm in Table 1.

Table 3: The concentrations of lycopene and β-carotene in papaya and orange juice using the predicted equations

[a] Sample	Absorbances At 450nm	Absorbances At 502nm	[b] Carotene concentration (μM)	[c] Lycopene concentration (μM)	Carotene concentration (μg/g)	Lycopene concentration (μg/g)
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 10g and the volume of hexane extract is 200ml;
[b] Calculated using equation (5); [c] Calculated using equation (6)
The results are the means of triplicate analysis with reported standard deviation (SD).

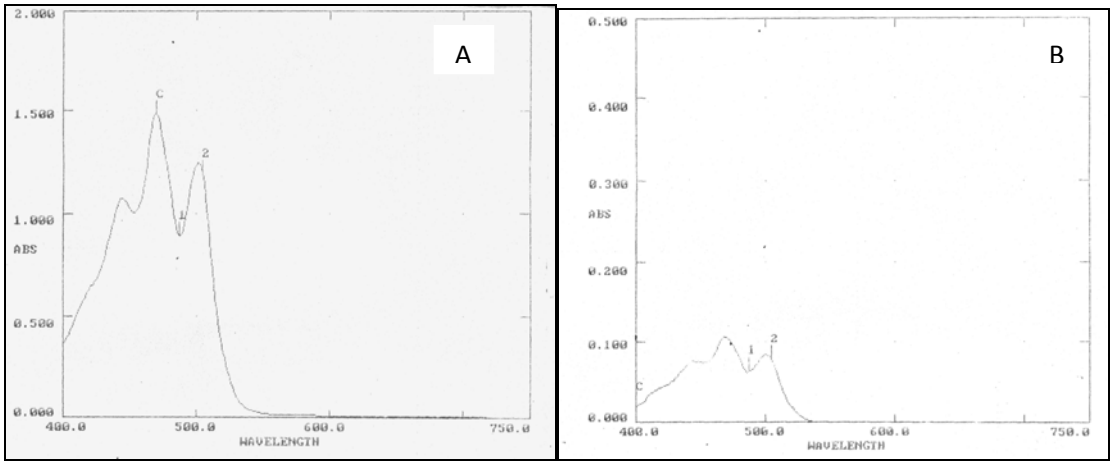

Figure 1: UV-Visible spectra of n-hexane extract of the lycopene/β-carotene mixture of tomato samples at dilution factors of (A) 1 (B) 16. Data measured on HEλI0Sα UV-Visible spectrophotometer.

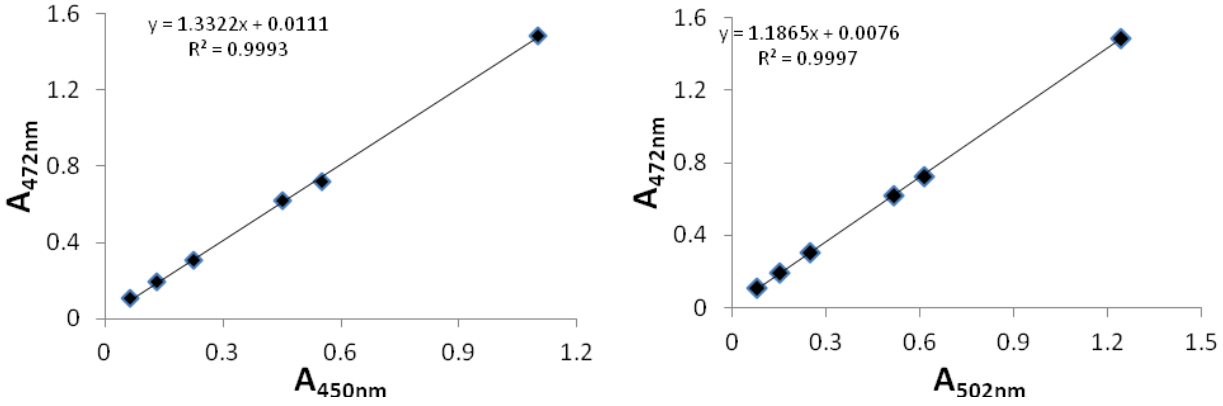

Figure 2: The correlations between the absorbances measured at 450nm and 502nm to that measured at 472nm for the hexane extract of the lycopene/β-carotene mixture of tomato samples

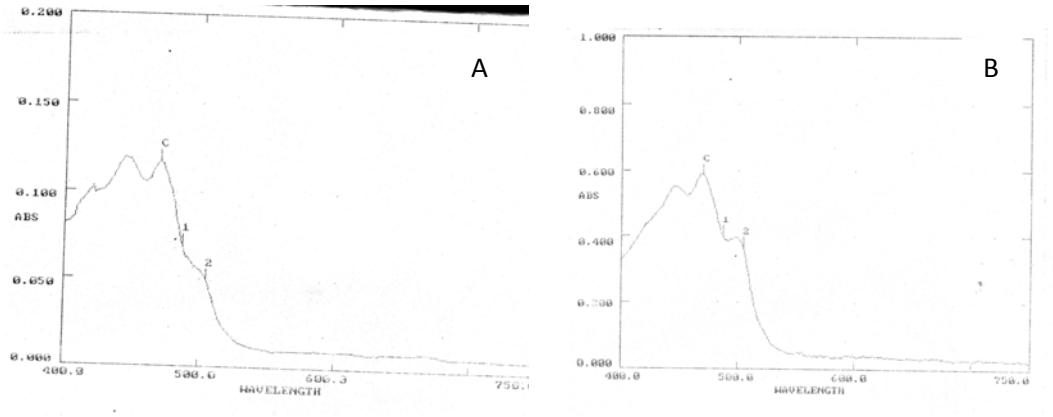

Figure 3: UV-Visible spectra of n-hexane extract of the lycopene/β-carotene mixture in samples of (A) orange juice (B) papaya. Data measured on HEλI0Sα UV-Visible spectrophotometer.