D. Separation and Determination of β-Carotene as Precursor of Vitamin A in Biological Samples

Recent publications concern the qualitative and quantitative determination of β-carotene by TLC. β-Carotene, pheophytin a, chlorophyll a and b, lutein, violaxanthin, and neoxanthin extracted from barley leaves were separated on HPTLC CN-coated plates using a mobile phase of chloroform–hexane–methanol (5:14:1) (R_f 0.83, 0.51, 0.41, 0.31, 0.19, 0.18, 0.13, respectively) (43). Typical chromatograms of chloroform extracts of barley leaves were also given.

Photoisomerization of individual all-*trans*-α- and β-carotene solutions produced several isomers each. Nyambaka and Ryley (44) described a TLC method to isolate and collect major isomers of β-carotene (13-*cis*-, all-*trans*-, 9-*cis*, and 7-*cis*). Two-dimensional TLC was done on calcium hydroxide plates with 1.2% acetone in petroleum ether as mobile phase. After extraction of the zones by TLC, the isomers were identified by their behavior in UV-Vis absorbance spectra. This method was applied to dark green leafy vegetables (Italian spinach, spring cabbage, and cowpea leaves). Identical conditions were used to develop and separate α- and β-carotene isomers by two-dimensional TLC. Expected isomers from the iodine-catalyzed reaction were neoisomers U and B for β-carotene and neoisomers U, W, and B for α-carotene. These isomers were detected by TLC in fresh and processed vegetables (spinach, cucumber, pickle, sweet potato, carrot) (45). β-Carotene was identified and separated from six other chloroplast pigments (46). Quantification of β-carotene and other pigments in spinach leaves was performed by scanning densitometry on the C_{18} layer at their wavelengths of maximum absorption (Fig. 3). β-Carotene and lutein were also identified and quantified in extracts from snail samples (Pennsylvania and Colorado strains of *Helisoma trivolvis* and *Biomphalaria glabrata*) (46).

The carotenoid composition, including β-carotene, of *Rosa canina* fruit was determined by TLC with densitometric analyses and also by HPLC. The peaks of the extracts obtained from the TLC densitograms were identified as β-carotene, lycopene, rubixanthin, β-chryptoxanthin, and zeaxanthin mixed with lutein (R_f 0.96, 0.90, 0.62, 0.53, and 0.32, respectively) on silica gel with 15% v/v acetone in petroleum ether. The distribution of these compounds was reproducible by TLC as well as by HPLC (47).

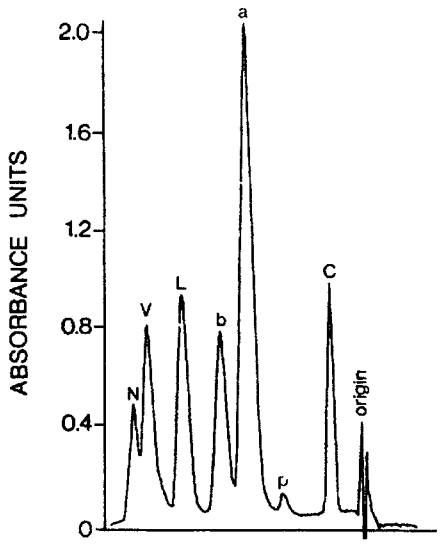

Figure 3 Reflectance densitogram at λ = 429 nm of a spinach leaf extract separated on a C-18 plate developed with petroleum ether–acetonitrile–methanol (2:4:4). N = neoxanthin, V = violaxanthin, L = lutein, b = chlorophyll b, a = chlorophyll b, p = pheophytins, C = β-carotene. (From Ref. 46.)