Some studies directed at assisting carotenoid research

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Data on carotenoids vary from laboratory to laboratory depending on the origin of results. However a reliable database is necessary for the use of nutritionists for recommendation of appropriate diets. The objective of this study was to focus on the problem areas of data generation and interpretation including mainly (i) sampling of raw materials (ii) identification and (iii) quantification of carotenoids. This was necessary as past Sri Lankan studies made these errors discussed below. The problems of sampling need to be overcome. Results showed that variation in carotenoid content was very large in crops that have no agricultural cultivars *e.g.* yellow-fleshed papaw, *Lasia spinosa* (kohila). As a result in such cases standard deviation (SD) cannot be calculated. On the other hand SD could be calculated in species with specific agricultural varieties (cultivars) *e.g.* carrot, pumpkin, sweet potato. In addition to genetic factors, studies showed that the carotenoid content in plant foods was affected by maturity and post-harvest carotenogenesis. These variables make it virtually impossible for a nutritionist to predict the retinol equivalent per meal/portion. In many cases data from spectra, thin layer chromatography (TLC), high performance liquid chromatography (HPLC), open column chromatography (OCC) and chemical tests were needed to be analysed in conjunction to obtain correct identification due to the following problems. HPLC peak enrichment with the isolates eluted from OCC of the same sample was important. But HPLC peak enrichment/retention time was not enough as different carotenoids co-chromatograph. TLC was useful to assess the number of hydroxy groups which was inversely proportional to the R_f only on activated plates run the full distance. Use of non-activated plate gave difficulties in identification of different hydroxylated carotenoids. Hydrocarbon carotenoids migrated close to the solvent front; R_f>0.9 on both activated and non-activated plate. Definition of plate characteristics, running the plate to the full extent and use of standards on the same plate is important. On the other hand different carotenoids gave the same R_f value. In iodine catalysed *cis/trans* isomerisation test, it was found that a baseline correction must be done with a blank containing the exact amount of iodine as the reaction mixture, only after isomerisation. In quantification, without a photodiode array facility, problems arose when λ_{max} values of carotenoids are different from each other. In the case of simple profiles the λ_{max} of each carotenoid can be scanned. This is not possible when the number is very high. Then a correction factor needs to be employed giving the ratio of absorbance at λ_{max} of that particular carotenoid and absorbance at 450 nm. In addition another correction factor needs to be applied for the extinction coefficient. Use of internal standard was necessary not only to correct for recovery but also for verification of authenticity of results even if there is auto-injection. In quantification by OCC the problem arises with unidentified carotenoids where $A^{1\%}_{1cm}$ is not known. Here $A^{1\%}_{1cm}$ of the known carotenoids which eluted close to the unknown compound in OCC can be used as they have very similar chemical properties. In OCC even though petroleum ether of B.P.60-80 °C was better than B.P. of 40-60 °C it was more difficult to flush out with nitrogen at later stages. Determination of the antioxidant capacity of carotenoids by using the standard $ABTS^{+\cdot}$ (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid cation free radical) diammonium salt method was difficult due to solubility problems of carotenoids. This could be overcome by using ethanol in place of phosphate buffer. Although the effect of concentration was not exactly linear, the results showed the trend of the antioxidant capacity of each carotenoid. There are many disagreements on carotenoid analyses results not only in Sri Lanka but also internationally. Reasons for some of these disagreements have been clarified.

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Professor MUS Sultanbawa Memorial Oration 2008
Gold Medal Award

The Professor MUS Sultanbawa Memorial Oration was delivered by Professor Priyani Paranagama of the Department of Chemistry, University of Kelaniya, on 9th May 2008 at Adamantane House. Her presentation was entitled "Exploring small molecule bioactive natural products: Selected examples from plant associated fungi, endolichenic fungi and essential oil bearing plants". The complete oration and other information about the event will be published in the September issue of Chemistry in Sri Lanka.