ELSEVIER

Food Chemistry 64 (1999) 269–275

Food Chemistry

Analytical, Nutritional and Clinical Methods Section

HPLC analysis of carotenoids in orange juice

A.M. Pupin[a,*], M.J. Dennis[b], M.C.F. Toledo[c]

[a]*Centro Pluridisciplinar de Pesquisas Químicas, Biológicas e Agrícolas (CPQBA), Universidade Estadual de Campinas, CP 6171 Campinas SP, CEP 13081-970, Brazil*

[b]*CSL Food Science Laboratory, Norwich Research Park, Colney, Norwich NR4 7UQ, UK*

[c]*Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, CP 6121, Campinas SP, CEP 13081-970, Brazil*

Received 6 June 1997; received in revised form and accepted 10 March 1998

Abstract

Lutein, zeaxanthin, β-cryptoxanthin, α-carotene and β-carotene have been determined in samples of Brazilian orange juice (*Citrus sinensis*). The concentrations found in factory-produced concentrates have been compared with those obtained on the Brazilian retail market and with authentic hand-squeezed juices. The analyses of the latter enabled a comparison of varieties to be made. A concentration range of 0.11–1.21 mg litre^{-1} was determined for total carotenoids with β-carotene, the most important source of Vitamin A, being found in the highest concentration in the Pera variety followed by Valência, Natal, Lima and Baía varieties. The total carotenoids present in samples of frozen concentrated orange juice (FCOJ) obtained from factories ranged from 0.26–0.48 mg litre^{-1}, while retail samples of this product contained slightly more (0.46–0.81 mg litre^{-1}). Frozen concentrated orange pulp-wash presented much lower concentrations, ranging from 0.04 to 0.08 mg litre^{-1} of total carotenoids. Fourteen samples of retail freshly-squeezed orange juice contained carotenoids ranging from 0.04 to 0.55 mg litre^{-1}, with only one sample out of the range found for authentic samples. This could be due to the addition of pulp-wash to this sample, in which undeclared sorbic acid was also detected.

1. Introduction

Brazil provides 80% of the world market of frozen concentrated orange juice (FCOJ) (Robards and Antolovich, 1994). Although there is a retail market for FCOJ in Brazil, freshly squeezed orange juice (FSOJ) sold in different packaging is gaining market share and various brands are now available. Pulp-wash is an industrially traded commodity. It is prepared, as the name suggests, as an aqueous extract of the pulp and enzymes are sometimes used to increase the effectiveness of this process. It is used in the formulation of soft drinks but is not normally sold to consumers directly. FSOJ commands a higher price than FCOJ or pulp-wash, and is retailed as a 100% natural juice obtained from fresh oranges without the addition of any preservatives, sugars, colorants or water.

Orange juice is one of a number of dietary sources of carotenoids and, indirectly, pro-vitamin A. Consumption of this vitamin has been correlated with a reduction in the incidence of certain cancers (Colditz et al., 1985; Olson, 1986; Bendich, 1989; Ziegler, 1989, 1991).

Fisher and Rouseff (1986) described the analysis of carotenoids (including α-carotene, β-carotene, and β-cryptoxanthin) in orange juice using HPLC. Quackenbush and Smallidge (1986) extended this work to include zeinoxanthin and separated it from β-cryptoxanthin. They measured α-carotene and *cis* and *trans*-β-carotene which substantially improved the reliability of information on vitamin A content in food. Philip et al. (1989) used a similar system to analyse several carotenoids in unsaponified orange juice with the objective of determining adulteration. In order to create peak patterns for computer pattern analysis, a series of 32 peaks were chosen as representative of authentic orange juice.

Recently, Rouseff et al. (1996) reported the use of a unique C-30 reversed-phase column to separate thirty-nine carotenoids, using a gradient of water, methanol and *tert*-butyl ether. According to the authors the main advantage of using this method was the improvement in resolution of the chromatographic system and the speed of analysis.

A limited amount of data is available concerning the analysis of carotenoids in orange juice and it is often the case that the varieties widely used in Brazil are not represented. Pera Rio (40.4%) is the most widely grown Brazilian variety with Natal (30.6%), Valência (17.3%),

* Corresponding author.

0308-8146/98/$—see front matter
PII: S0308-8146(98)00095-8

Hamlin (7.8%) and others (3.9%) also grown (Steger, 1990). Data on the carotenoid content of Brazilian orange varieties, either from the fruit or as the processed product, are limited. This paper reports the concentrations of α-carotene, β-carotene, lutein, zeaxanthin and β-cryptoxanthin in these products.

2. Material and methods

2.1. Standards

Sudan I (dye), α-carotene, β-carotene and lutein were obtained from Sigma. Zeaxanthin and β-cryptoxanthin were obtained from Apin Chemicals (Oxon, UK).

Lutein, α-carotene, β-carotene, zeaxanthin and β-cryptoxanthin (5 mg each) were dissolved in chloroform containing 0.1% butylated hydroxytoluene (BHT) and the volume made up to 25 ml in an ambered flask. This solution was prepared monthly. A working standard was prepared by dilution of the stock standard (2.5 ml) with acetonitrile (100 ml). Chromatographic standards (prepared daily) were obtained by diluting appropriate amounts of the working standards and adding 100 µl of Sudan I (50.0 mg litre^{-1} dissolved in acetonitrile). The concentrations for the carotenoid standards ranged from 0.03 to 0.4 mg litre^{-1}.

To calculate the concentration of each carotenoid standard solution, 5 ml were evaporated under nitrogen and dissolved in the appropriate solvent according to the method described by Hart and Scott (1995) and the UV absorbance measured. The concentration was determined using the specific extinction coefficient of each carotenoid.

2.2. Quantitative analysis

The concentration of carotenoids in the samples was determined by comparison with external standards (lutein, α-carotene, β-carotene, zeaxanthin and β-cryptoxanthin) taking account of the response of the injection standard (Sudan I). No correction for recovery was applied to the data.

2.3. Recovery studies

Reagent blank controls were analysed for the presence of possible interference and no extraneous peaks were observed. In order to ensure that the carotenoids were correctly identified, samples were spiked with several concentrations of each carotenoid (0.07 to 0.53 mg litre^{-1}). Recovery of carotenoids was measured for quality control purposes.

2.4. Quality control

An In-House Reference Material (IHRM) was used during the analysis to ascertain that the method was under control and to determine the repeatability of the method. A sample of orange juice (1 litre) was well mixed and divided into vials containing 12 ml each and frozen at −20°C until analysis.

2.5. Samples

Authentic samples of oranges from different varieties, as well as commercial concentrated orange juice and pulp-wash, were collected from processing plants in the State of São Paulo (Brazil). Retail samples (frozen concentrated orange juice and freshly squeezed orange juice) were purchased from supermarkets in the metropolitan area of Campinas (State of São Paulo) during the years of 1995/1996.

2.6. Sample preparation

Citrus fruit were hand-squeezed and the juices filtered through a stainless steel sieve (1.25 mm). No allowance was made for differences in °Brix of these samples. Frozen concentrated orange juice and frozen concentrated pulp-wash were diluted to 12° Brix with Millipore water and results are expressed on this basis. Retail freshly squeezed orange juices were sieved before analysis. All of the samples were stored at −20°C.

2.7. Sample analysis

Samples of orange juice (5 ml) were extracted with ethyl acetate (3×50 ml) containing BHT (0.004%). The organic phase was transferred through anhydrous sodium sulphate (50 g) and collected in an ambered round-bottom flask. To the aqueous residue 50 ml of methanol was added (containing 0.004% BHT) followed by 100 ml of 1M NaCl. The solution was well mixed and further extracted with ethyl acetate (75 and 25 ml, containing 0.004% BHT). The ethyl acetate fractions were then transferred through the sodium sulphate and combined with the previous extracts. Finally the sodium sulphate was washed with a further 50 ml of ethyl acetate (0.004% BHT). The pooled ethyl acetate was evaporated to dryness in a rotary evaporator at 40°C. The extract was transferred quantitatively to a 10 ml volumetric flask using portions of 1.5 ml of mobile phase (acetonitrile:methanol:1,2-dichloroethane, 60:35:5, v/v/v). The injection standard (100 µl, Sudan I, 50 mg litre^{-1} in acetonitrile) was added and the volume was made up to 10 ml.

2.8. High performance liquid chromatography (HPLC)

The HPLC apparatus consisted of a Waters 625 LC System, equipped with an autosampler Gilson 231 XL and a Spectra Focus UV-Vis detector (Spectra Physics). A 100 µl loop was used for injection. Solvents were HPLC

grade. The mobile phase was a ternary mixture of acetonitrile:methanol:1,2-dichloroethane (60:35:5, v/v/v) to which 0.1% BHT, 0.1% triethylamine and 0.05 M of ammonium acetate (in methanol) was added (Hart and Scott, 1995). The column was a C18 Vydac 201TP54 5 μm (250×4.6 mm id., Vydac) with a guard-column Alltima C18 5 μm (7.5×4.6 mm id., Alltech). The column was kept at room temperature (about 22°C) and the flow rate was 1 ml min^{-1}. The wavelength was adjusted to 450 nm. The peak areas were measured using a Millennium Software v. 2.0 (Waters). Peak identity was confirmed by a Spectra Focus Scanning Detector (Spectra Physics). This equipment takes spectra (380–520 nm) from three points at different times across the HPLC peak and compares these spectra. If these spectra are identical, the peak is considered pure, i.e. no interferents are present.

3. Results and discussion

3.1. Sample preparation and high performance liquid chromatography

In order to avoid possible degradation and/or formation of artefacts the samples were extracted directly with ethyl acetate without saponification. The use of ethyl acetate prevented the problem of emulsion formation which may occur when using other solvents. According to De Ritter and Purcell (1981) the main reason for saponification is a gross purification of the carotenoids, which removes neutral fats, fatty acids and esters present in the juice besides simplifying the chromatogram (Fisher and Rouseff, 1986). This approach has been adopted by others analysing citrus juices, e.g. Rouseff et al. (1992), who analysed carotenoids in red grapefruit cultivars, without saponification, using a mixture of hexane, acetone and ethanol, as extraction solvent.

The liquid chromatographic procedure uses a non-aqueous reverse phase (NARP) system which included triethylamine, ammonium acetate and BHT to prevent the degradation of carotenoids on column. NARP chromatography has been used by several authors to analyse carotenoids in different foods (Nelis and De Leenheer, 1983; Bushway, 1986; Fisher and Rouseff, 1986; Heinonen et al., 1989; Handelman et al., 1992; Chen, 1992; Epler et al., 1993; Hart and Scott, 1995; Lin and Chen, 1995). However, the use of modifiers in the mobile phase to avoid degradation of the carotenoids is a recent innovation. Handelman et al. (1992) used ammonium acetate (0.01%) in the mobile phase while Epler et al. (1993) included both ammonium acetate and triethylamine in their solvent mixture. This gave a 94% recovery for the carotenoids studied. Hart and Scott (1995) added a third component to the mobile phase (0.1% BHT) in order to achieve approximately 100% recovery. It has been suggested that the main function of ammonium acetate and triethylamine in the mobile phase is to minimize the effects of the acidity provided by free sylanol groups on the HPLC column (Handelman et al., 1992; Epler et al., 1993; Hart and Scott, 1995).

In order to avoid degradation of carotenoids during extraction, a maximum of two samples were extracted simultaneously in subdued lighting and immediately injected in the HPLC system. Tests conducted, extracting more than two samples at once, resulted in lower recovery levels.

3.2. Peak purity and identification

A Scanning UV detector was used to identify and characterise the carotenoids determined in this study. The peaks were identified by comparison of the spectra with standards and literature values and by the retention time standards. The spectra obtained showed no indication of coeluting and all agreed with those reported in the literature (De Ritter and Purcell, 1981). This technique has been used by several authors to identify carotenoids in orange juice, grapefruit and in green vegetables (Fisher and Rouseff, 1986; Chen, 1992; Rouseff et al., 1992; Rouseff et al., 1996). A typical chromatogram of carotenoids in orange juice is shown in Fig. 1.

3.3. Internal standard

There are no carotenoid-like materials commercially available which would be suitable for use as an internal standard. It was therefore decided to use a dye (Sudan I)

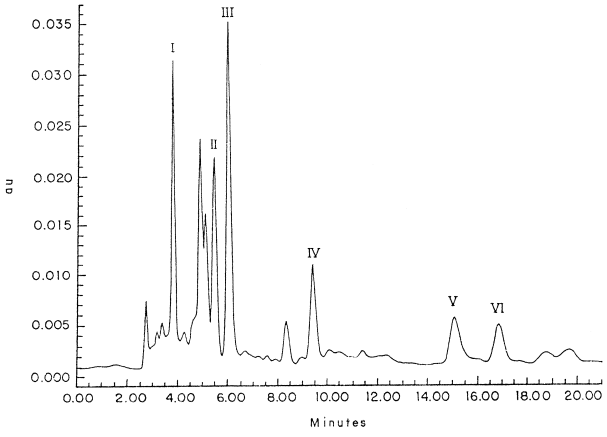

Fig. 1. HPLC chromatogram of carotenoids in an authentic sample of orange juice: I Sudan I, II lutein, III zeaxanthin, IV β-cryptoxanthin, V α-carotene, VI β-carotene. Column C18 Vydac 5 μm (250×4.6 mm i.d.), pre-column C18 Alltech 5 μm (7.5×4.6 mm i.d.), mobile phase acetonitrile:methanol:1,2 dichlorethane (60:35:5, v/v/v) added with 0.1% BHT, 0.1% TEA, 0.05 M ammonium acetate in methanol. Flow 1.0 ml min^{-1}, Vis 450 nm.

Table 1
Mean and coefficient of variation of the IHRM for the determination of carotenoids

	Lutein	Zeaxanthin	β-Cryptoxanthin	α-Carotene	β-Carotene
Mean (mg litre^{-1})	0.06	0.10	0.03	0.02	0.03
CV (%)	19.8	17.2	13.4	17.7	16.6

Twenty-one samples were analysed.

for this purpose. Since this material does not have the same susceptibility to oxidation as carotenoids, it was used solely to quantify the injection and so was added at the end of the extraction process. Sudan I [1-(phenylazo)-2-naphthalenol] has previously been used as an internal standard by Quackenbush and Smallidge (1986) to determine pro-vitamin A in food and by Philip et al. (1989) to detect adulteration of orange juice with added carotenoids.

3.4. Linearity of response and limit of detection

All carotenoids showed a linear response within the range studied: 0.03 to 0.4 mg litre^{-1} ($r = 0.999$ or better). The following limits of detection were estimated using a signal to noise ratio of 3: lutein, zeaxanthin β-cryptoxanthin 0.01 (mg litre^{-1}) α and β-carotene 0.02 (mg litre^{-1}).

3.5. Quality control

Table 1 shows the precision achieved from 21 analyses of an IHRM determined over the course of the study. An average coefficient of variation (CV) of 16.9% was obtained for the five carotenoids considered. Hart and Scott (1995) determined a CV ranging from 5.6 to 11.8% and 4.9 to 10.8%, respectively, for short term and long term reproducibility in a mixture of vegetables. Epler et al. (1993) used a similar system to ascertain that the method was in control and to determine the reproducibility of the method to quantify carotenoids in human serum and food. In their analyses the CV for the "low quality control" and "normal quality control" ranged from 3.1 to 14.0% and 2.1 to 15.2%, respectively. Heinonen et al. (1989) obtained an average of 10% in the CV of triplicate analyses with values ranging from 0.8 to 44% depending on the amount and complexity of the carotenoids in the food item.

Table 2
Recoveries levels for carotenoids in orange juice

Compound	n	Amount added (mg litre $^{-1}$)	Mean recovery % (SD)
Lutein	10	0.09–0.53	78.1 (11.2)
Zeaxanthin	10	0.07–0.43	70.8 (12.8)
β-Crypthoxantin	10	0.10–0.46	66.9 (11.6)
α-Carotene	10	0.09–0.47	77.6 (11.2)
β-Carotene	10	0.10–0.53	61.3 (13.3)

n = number of samples.

Table 2 shows the recoveries obtained from the analysis of fortified reagent blanks. The average value was 71% for the five carotenoids studied. Limited information is available on recovery studies for carotenoids in orange juice. Fisher and Rouseff (1986) obtained a mean recovery of 80% for β-carotene and Quackenbush and Smallidge (1986) recovered 96% of β-carotene added.

4. Authentic Samples

4.1. Hand-squeezed orange juice

The carotenoid content of authentic samples of hand squeezed orange juices is given in Table 3. The different varieties investigated showed some variation in carotenoid concentrations. The total amount of carotenoids ranged from 0.11 to 1.21 mg litre^{-1}. The variety Pera Rio contained the highest amount of carotenoids ranging from 0.63 to 1.21 mg litre^{-1}, while concentrations in the other varieties were fairly similar. In all samples zeaxanthin was present in the highest concentration. β-carotene, the main source of pro-vitamin A, was found in higher concentrations in the Pera variety.

These results are in reasonable agreement with those described by Heinonen et al. (1989) and Stewart (1977a), although the levels found were lower than the ones found by Reeder and Park (1975). As demonstrated by Stewart (1977a) the amount of carotenoids increases with the fruit maturity and varies according to the type. For example, in his study the content of β-carotene in the Valência variety ranged from 0.008 (7 October) to 0.089 mg litre^{-1} (30 March) and, for the same period, the Murcott variety ranged from 0.027 to 2.38 mg litre^{-1}.

4.2. Frozen concentrated orange juice (FCOJ) and frozen concentrated orange pulp-wash (FCOPW)

The carotenoid content of FCOJ and of FCOPW is detailed in Table 4. Again the variety Pera Rio (in FCOJ) contained higher amounts of carotenoids when compared to the other two samples. The results for total carotenoids in FCOJ (0.26 to 0.48 mg litre^{-1}) are in reasonable agreement with those previously described for commercial FCOJ (Stewart, 1977b; Quackenbush and Smallidge, 1986).

Low concentrations of total carotenoids were found in FCOPW with a maximum observed of 0.08 mg

Table 3
Levels of carotenoids (mg litre^{-1}) in authentic (hand-squeezed) orange juice

Variety	Lutein	Zeaxanthin	β-Cryptoxanthin	α-Carotene	β-Carotene	Total
Pera Rio ($n=5$)	0.14–0.23	0.30–0.53	0.10–0.19	0.04–0.18	0.05–0.08	0.63–1.21
$\bar{x}$	0.18	0.39	0.13	0.09	0.07	0.86
Natal ($n=4$)	0.04–0.06	0.06–0.12	0.01–0.03	0.03–0.04	0.02–0.03	0.18–0.28
$\bar{x}$	0.05	0.09	0.02	0.03	0.03	0.22
Valência ($n=3$)	0.06–0.07	0.04–0.10	0.02	0.03–0.07	0.02–0.05	0.17–0.31
$\bar{x}$	0.07	0.08	0.02	0.05	0.04	0.25
Hamlin ($n=3$)	0.03–0.08	0.06–0. 27	0.02–0.06	nd–0.02	nd–0.02	0.11–0.45
$\bar{x}$	0.05	0.16	0.04	0.01	0.01	0.27
Baía	0.05	0.04	0.08	nd	0.02	0.19
Lima	0.08	0.10	0.01	0.02	nd	0.21
Pera Coroa	0.08	0.15	0.05	0.05	0.04	0.37

nd = not detected (<0.02 mg litre^{-1}).
n = number of analysed samples.
Results not corrected for recovery.

Table 4
Levels of carotenoids (mg litre^{-1}) in authentic samples of frozen concentrated orange juice (FCOJ) and in frozen concentrated orange pulp wash (FCOPW), both diluted to 12° Brix

Samples		Lutein	Zeaxanthin	β-Cryptoxanthin	α-Carotene	β-Carotene	Total
FCOJ	1[a]	0.06	0.09	0.05	0.02	0.04	0.26
	2[b]	0.10	0.22	0.08	0.03	0.05	0.48
	3[c]	0.09	0.14	0.08	0.02	0.04	0.37
	$\bar{x}$	0.08	0.15	0.07	0.02	0.04	0.37
	SD	0.02	0.07	0.02	0.01	0.01	0.11
FCOPW ($n=2$)		0.01–0.03	0.02–0.04	0.01	nd	nd	0.04–0.08

[a] Variety Hamlin.
[b] Variety Pera Rio.
[c] Mixture of several varieties.
nd = not detected (<0.02 mg litre^{-1}).
n = number of analysed samples.
Results not corrected for recovery.

litre^{-1}. Neither α nor β-carotene was detected in the samples analysed. These levels are much smaller than those found in hand-squeezed juices or FCOJ. As FCOPW is depleted in colour its addition to orange juice will result in a reduction in the colour intensity. It is possible that colorants such as synthetic β-carotene might be added to mask this adulteration. If this is the case, then differences in the ratio of the individual carotenoids might provide an indication of this practice.

5. Retail samples

5.1. Retail frozen concentrated orange juice (RFCOJ)

The amount of carotenoids ranged from 0.46 to 0.81 mg litre^{-1} in RFCOJ (Table 5). These concentrations are, if anything, larger than those obtained for the factory samples (FCOJ). Stewart (1977b) and Quackenbush and Smallidge (1986) found similar concentrations in commercial samples of FCOJ. As there was no evidence of a reduction in the total amount of carotenoids it can be assumed that the retail samples analysed here were not adulterated with pulp-wash.

5.2. Retail freshly squeezed orange juice (RFSOJ)

The amount of carotenoids ranged from 0.04 to 0.55 mg litre^{-1} in RFSOJ (Table 6). With the exception of one sample, the concentrations observed were in accordance with those anticipated from authentic samples (Table 3) and gave no indication of the addition of pulp-wash or other diluents. The profile of the carotenoids matched that found for authentic samples with zeaxanthin found in the highest concentration and

Table 5
Levels of carotenoids (mg litre^{-1}) in retail samples of frozen concentrated orange juice (FCOJ), diluted to 12° Brix

	Lutein	Zeaxanthin	β-Cryptoxanthin	α-Carotene	β-Carotene	Total
$n=3$	0.09–0.12	0.19–0.45	0.08–0.11	0.04–0.05	0.06–0.08	0.46–0.81
$\bar{x}$	0.11	0.30	0.10	0.04	0.07	0.62

n = number of samples analysed.
Results not corrected for recovery.

Table 6
Levels of carotenoids (mg litre^{-1}) in retail samples of freshly squeezed orange juice

	Lutein	Zeaxanthin	β-Cryptoxanthin	α-Carotene	β-Carotene	Total
$n=14$	0.02–0.13	0.02–0.20	nd[a]–0.13	nd[b]–0.04	nd[b]–0.05	0.04–0.55
$\bar{x}$	0.08	0.11	0.06	0.03	0.04	0.32

nd [a] = not detected (<0.01 mg litre^{-1}).
nd [b] = not detected (<0.02 mg litre^{-1}).
n = number of samples analysed.Results not corrected for recovery.

β-carotene present in similar amounts to the authentic hand-squeezed juice. It might be anticipated that the variability of industrially-processed product would be less than that found for hand-squeezed samples because the industrial product will contain a mixture of a large number of fruits prepared in a highly controlled procedure. This expectation proves to be correct. The average concentration of carotenoids in the hand-squeezed juice was 0.42 mg litre^{-1} (SD = 0.30) whereas the retail FSOJ (even including the anomalous result for one sample) gave 0.32 mg litre^{-1} (SD = 0.13).

6. Conclusion

The usefulness of the procedure in determining adulteration of retail samples of orange juice can be seen from the data obtained for one sample. The concentration of total carotenoids in this sample (0.06 mg litre^{-1}) was considerably less than that found for hand squeezed samples (average of 0.42 mg litre^{-1}) and from the vast majority of industrially produced samples (average of 0.62 mg litre^{-1} for FCOJ). It is apparent, therefore, that the sample has been extended, perhaps with a material such as pulp-wash, (which contained 0.06 mg litre^{-1} of total carotenoids) or with sugar and water. Further evidence that this sample was not an authentic pure orange juice was provided by the discovery of sorbic acid in it.

Acknowledgements

Financial support for A. M. Pupin from CAPES Process no. BEX 0206/95-1 is gratefully acknowledged. We would like to thank Citrosuco and Cutrale for supplying the authentic samples of oranges.

References

Bendich, A. (1989) Carotenoids and the immune response. *J. Nutr.* **119**, 112–115.
Bushway, R. J. (1986) Determination of α- and β-carotene in some raw fruits and vegetables by high-performance liquid chromatography. *J. Agric. Food Chem.* **34**, 409–412.
Chen, B. H. (1992) Studies on the stability of carotenoids in Garland chrysanthemum (*Ipomea spp.*) as affected by microwave and conventional heating. *J. Food Protec.* **55**(4), 296–300.
Colditz, G. A., Branch, L. G., Lipnick, R. J., Willett, W. C., Rosner, B., Posner, B. M. and Hennekens, H. (1985) Increased green and yellow vegetable intake and lowered cancer deaths in an elderly population. *Am. J. Clin. Nutr.* **41**, 32–36.
De Ritter, E. and Purcell, A. E. (1981) Carotenoid analytical methods. In *Carotenoids as Colorants and Vitamin A Precursors*, ed. J. C. Bavernfeind. Academic Press, New York, pp. 815–883.
Epler, K. S., Ziegler, R. G. and Craft, N. E. (1993) Liquid chromatographic method for the determination of carotenoids, retinoids and tocopherols in human serum and in food. *J. Chromatogr.* **619**, 37–48.
Fisher, J. F. and Rouseff, R. L. (1986) Solid phase extraction and HPLC determination of β-cryptoxanthin and α and β-carotene in orange juice. *J. Agric. Food Chem.* **34**, 985–989.
Handelman, G. J., Shen, B. and Krinsky, N. I. (1992) High resolution analysis of carotenoids by high-performance liquid chromatography. *Methods Enzymol.* **213**, 336–346.
Hart, D. J. and Scott, K. J. (1995) Development and evaluation of an HPLC method for the analysis of carotenoids in foods, and the measurement of the carotenoid content of vegetables and fruits commonly consumed in the UK. *Food Chem.* **54**, 101–111.
Heinonen, M. I., Ollilainen, V., Linkola, E. K., Varo, P. T. and Koivistoinen, P. E. (1989) Carotenoids in Finnish foods: vegetables, fruits, and berries. *J. Agric. Food Chem.* **37**, 655–659.
Lin, S. D. and Chen, A. O. (1995) Major carotenoids in juices of ponkan mandarin and liucheng orange. *J. Food Biochem.* **18**, 273–283.
Nelis, H. J. C. F. and De Leenheer, A. P. (1983) Isocratic non aqueous reversed-phase liquid chromatography of carotenoids. *Anal. Chem.* **55**, 270–275.
Olson, J. A. (1986) Carotenoids, vitamin A and cancer. *J. Nutr.* **116**, 1127–1130.
Philip, T., Chen, T.-S. and Nelson, D. B. (1989) Detection of adulteration of California orange juice concentrates with externally

added carotenoids by liquid chromatography. *J. Agric. Food Chem.* **37**, 90–95.

Quackenbush, F. W. and Smallidge, R. L. (1986) Non aqueous reverse phase liquid chromatographic system for separation and quantitation of pro vitamins A. *J. Assoc. Off. Anal. Chem.* **69**(5), 767–772.

Reeder, S. K. and Park, G. L. (1975) A specific method for the determination of provitamin A carotenoids in orange juice. *J. Assoc. Off. Anal. Chem.* **58**(3), 595–598.

Robards, K. and Antolovich, M. (1994) Application of chromatography and pattern recognition to quality assessment of orange juice. *Chem. Austr.* **61**, 392–395.

Rouseff, R. L., Sadler, G. D., Putnam, T. J. and Davis, J. E. (1992) Determination of β-carotene and other hydrocarbons carotenoids in red grapefruit cultivars. *J. Agric. Food Chem.* **40**, 47–51.

Rouseff, R. L., Raley, L. and Hofsommer, J.-J. (1996) Application of diode array detection with a C-30 reversed phase column for the separation and identification of saponified orange juice carotenoids. *J. Agric. Food Chem.* **44**, 2176–2181.

Steger, S. (1990) Jahre Citrusverarbeitung Brasilien. *Flüss. Obst* **57**, 324–331.

Stewart, I. (1977a) Pro vitamin A and carotenoid content of citrus juices. *J. Agric. Food Chem.* **25**(5), 1132–1137.

Stewart, I. (1977b) High performance liquid chromatographic determination of pro vitamin A in orange juice. *J. Assoc. Off. Anal. Chem.* **60**(1), 132–136.

Ziegler, R. G. (1989) A review of epidemiologic evidence that carotenoids reduce the risk of cancer. *J. Nutr.* **119**, 116–122.

Ziegler, R. G. (1991) Vegetables, fruits and carotenoids and the risk of cancer. *Am. J. Nutr.* **53**, 251S–259S.