PERGAMON

Available online at www.sciencedirect.com
SCIENCE DIRECT®

Phytochemistry 62 (2003) 939–947

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Identification and quantification of carotenoids, tocopherols and chlorophylls in commonly consumed fruits and vegetables

Jennifer Burns[1], Paul D. Fraser, Peter M. Bramley*

School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey TW20 0EX, UK

Received 30 August 2002; received in revised form 14 November 2002

Abstract

The carotenoid, tocopherol and chlorophyll metabolic profiles and content of a selection of fruits and vegetables found commonly in the diet, have been determined using a rapid RP-HPLC technique with on-line PDA detection. Information gathered from the screening of secondary plant metabolites is vital for the accurate determination of the dietary intake of these micro-nutrients, and in the development of comprehensive food tables. Determination of basal levels is also necessary for the rational engineering of health-promoting phytochemicals in food crops. In addition this approach can also be applied to the routine screening of products to determine metabolic differences between varieties and cultivars, as well as between genetically modified and the corresponding non-genetically modified tissue.

Keywords: Metabolic profile; Carotenoid; Tocopherol; Chlorophyll

---

1. Introduction

Health professionals have consistently advocated the daily consumption of five portions of fruit and vegetables as a means of protecting against the pathogenesis of degenerative diseases including coronary heart disease, cancers and other free radical-mediated conditions (Kritchevsky, 1999).

Fruit and vegetables contain a wide range of compounds including the antioxidant vitamins C and E, minerals, phenolics and carotenoids. Carotenoids are natural pigments, providing orange, yellow, red and purple colours throughout the natural world. These pigments are produced by all higher plants, along with some bacteria and algae (Britton et al., 1995). They are derived from the terpenoid family and are biosynthetically related to other secondary metabolites such as tocopherols and ubiquinones (Fig. 1 and Table 1). Within plant tissue they are associated with photosynthetic membranes and are involved in photoprotection and light energy assimilation (Britton et al., 1995).

Carotenoids possess a range of important and well-documented biological activities. They are potent antioxidants and free radical scavengers (Grassmann et al., 2002), and can modulate the pathogenesis of cancers (van Poppel and Goldbohm, 1995) and coronary heart disease (Kritchevsky, 1999). Lycopene intake in particular is associated with a decreased incidence of prostate cancer (Giovannucci, 1999), while high levels of β-carotene are correlated with a reduction in the risk of developing lung cancer (Block et al., 1992). A number of carotenoids including α-carotene, β-carotene and β-cryptoxanthin have pro-vitamin A activity, since they are converted to retinal by mammals. This role is of particular importance, especially in developing countries where the dietary deficiency of vitamin A can lead to blindness and premature childhood mortality (Mayne, 1996). The xanthophylls (oxo-carotenoids), lutein and zeaxanthin are also known to provide protection against age-related macular degeneration, mediated by their ability to quench single oxygen and blue light in the retina (Landrum and Bone, 2001).

---

* Corresponding author. Tel.: +44-1784-443555; fax: +44-1784-430100.

E-mail address: p.bramley@rhul.ac.uk (P.M. Bramley).

[1] Present address: Food Standards Agency, 125 Kingsway, London, WC2B 6NH.

0031-9422/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0031-9422(02)00710-0

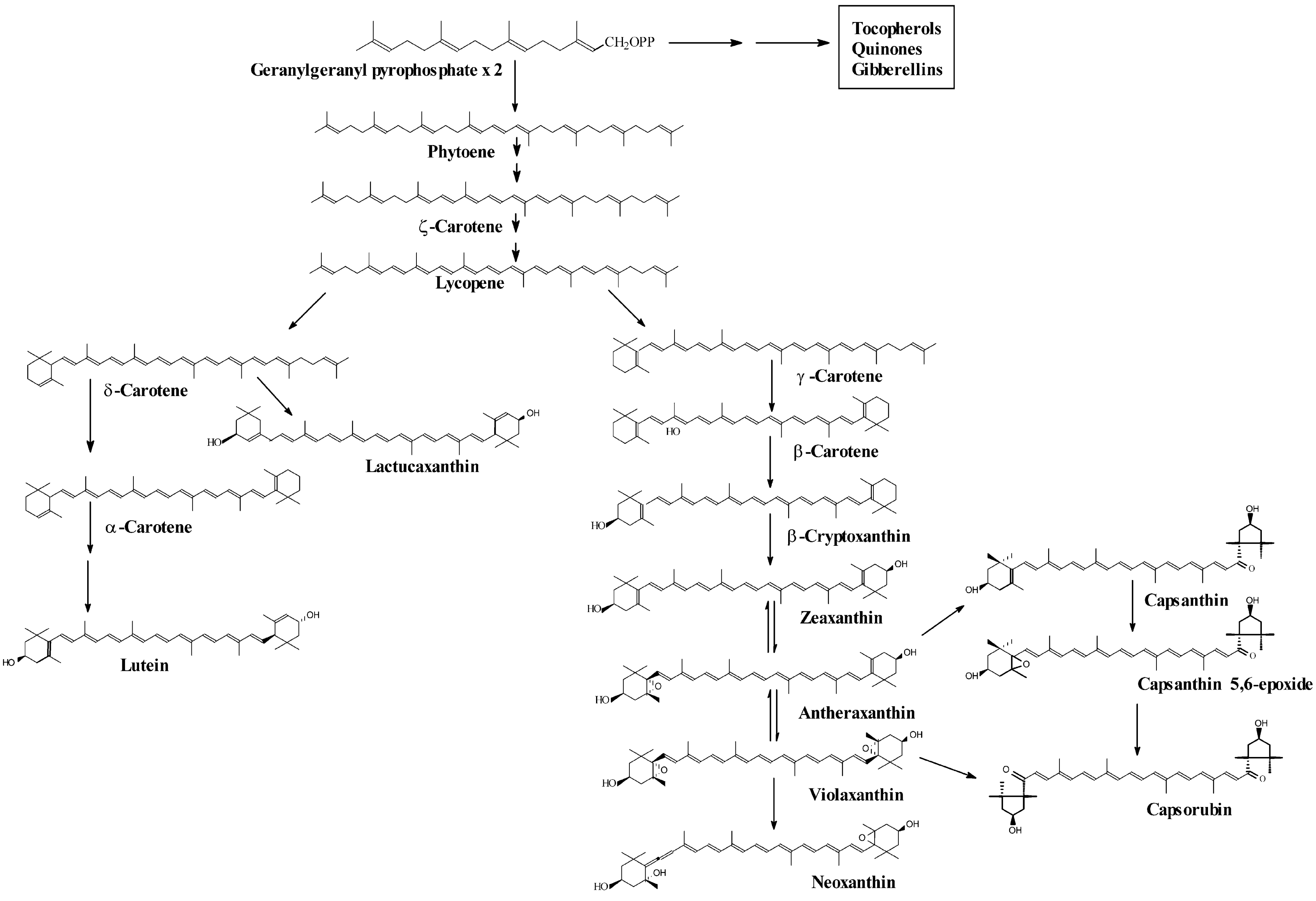

Fig. 1. Simplified pathway of carotenoid biosynthesis.

Table 1
Trivial and systematic nomenclature of carotenoids[a]

Trivial name	Systematic name
Phytoene	7,8,11,12,7′,8′11′,12′-Octahydrolycopene
ζ-Carotene	7,8,7′,8′-Tetrahydrolycopene
δ-Carotene	(6*R*)-ε-ψ-Carotene
α-Carotene	(6*R*)-β-ε-Carotene
Lactucaxanthin	(3*S*, 6*S*, 3′*S*, 6*S*)-)-ε-ε-Carotene-3,3′-diol
Lutein	(3*R*, 3′*R*, 6′*R*)- β-ε-Carotene-3,3′-diol
γ-Carotene	β-ψ-Carotene
β-Carotene	β-β-Carotene
β-Cryptoxanthin	(3*R*)-β-β-Carotene
Zeaxanthin	(3*R*, 3′*R*)-β-β-Carotene-3,3′-diol
Antheraxanthin	5,6-Epoxy-5,6-dihydro-β-β-carotene-3,3′-diol
Violaxanthin	(3*S*, 5*R*, 6*S*, 3′*S*, 5′*R*, 6′*S*)-5,6,5′,6′-Diepoxy-5,6,5′,6′-tetrahydro-β-β-carotene
Neoxanthin	(3*S*, 5*R*, 6*R*, 3′*S*, 5′*R*, 6′*R*)-5′,6′-Epoxy-6,7-didehydro-5,6,5′,6′-tetrahydro-β-β-carotene-3,5,3′-triol
Capsanthin	(3*S*, 2′*S*)-3,1′,2′-Trihydroxy-3′,4′-didehydro-1′,2′-dihydro-β-ψ-carotene-4-one
Capsanthin 5,6-epoxide	5,6-Epoxy-3,3′-dihydroxy-5,6-dihydro-β,χ-carotene-6′-one
Capsorubin	(3*S*, 5*R*, 3′*R*, 5′*R*)-3,3′-Dihydroxy-χ,χ-carotene-6,6′-dione

[a] Britton et al., 1995.

Significant advancement in the metabolic engineering of health-promoting phytochemicals (such as carotenoids) into food crops has been achieved. For example in rice endosperm the carotenoid pathway has been introduced resulting in the engineering of 'Golden Rice' (Ye et al., 2000), while in tomato both β-carotene (Römer et al., 2000) and lycopene (Fraser et al., 2002) content has been elevated. In order to extend these approaches to other stable food crops it is vital that basal levels are determined to facilitate future rational engineering.

Their wide distribution throughout the plant kingdom means that carotenoids are a ubiquitous part of the diet, however this is not represented in the current food tables prepared by the USDA (Holden et al., 1999). Little standardized information is available on the content of individual carotenoids in foods, with data often presented as 'total carotenoids' or as pro-retinal equivalents. However, the development of a polymeric C_{30} stationary phase has enabled the separation of geometric isomers of common carotenoids (Sander et al., 1994; Fraser et al., 2000). These improvements will lead to more accurate assessments being made of the content of carotenoids in both individual produce and the total diet (Lessin et al., 1997). The most frequently used analytical technique presently used to provide a global profile of metabolites present in a given tissue is GC–MS (Fiehn et al., 2000). Important plant products such as carotenoids are however, not amenable to GC separation and often do not ionise efficiently preventing MS detection.

This paper describes the application of robust and established extraction and HPLC techniques to the screening of carotenoids, along with tocopherols and chlorophylls, in a selection of commonly consumed fruits and vegetables. These data may be used in the development of comprehensive food tables, or in the investigation of differences in the metabolic profile of tissues.

2. Results and discussion

Ten common fruits and vegetables (Table 2) were selected and the content and profile of several plant

Table 2
Foods analysed

Common name	Latin name	Country of origin	Preparation
Lettuce	*Lactuca sativa* L.	Spain	White stalk removed
Broccoli	*Brassica oleracea* L.	Holland	Tough stalk removed
Mango	*Magnifera indica* L.	Costa Rica	Peeled and de-stoned
Red pepper	*Capsicum annuum* L.	The Netherlands	De-seeded
Yellow pepper	*Capsicum annuum* L.	Israel	De-seeded
Green pepper	*Capsicum annuum* L.	The Netherlands	De-seeded
Carrot	*Daucus carota* L.	England	Peeled
Swede	*Brassica rapa* L.	Scotland	Peeled
Sweet potato	*Ipomoea batatas* L.	USA	Peeled
Tomato	*Lycopersicon esculentum* Mill.	Spain	None

metabolites investigated. In addition to carotenoids, α-tocopherol and γ-tocopherol along with the chlorophylls *a* and *b*, were identified and quantified in this study.

Diverse metabolic profiles were observed in extracts of the products investigated (Figs. 2 and 3, Table 3). The green vegetables, lettuce, broccoli and green pepper all show similar elution profiles. They all contain high levels of both chlorophyll *a* and *b*, along with the xanthophylls violaxanthin and lutein, and two isomers of β-carotene: eluting at ca. 28 min is all *trans* β-carotene, closely followed by the isomer 9-*cis* β-carotene. Red pepper is characterized by the presence of two carotenoids, capsanthin and capsorubin. A range of fatty acid esters, particularly of capsanthin, is also found, eluting between 25 and 35 min (Breithaupt and Schwack, 2000). It is reported that the most common esters are between capsanthin and capsorubin, and the saturated lauric (C12:0), myristic (C14:0) and palmitic (C16:0) fatty acids (Breithaupt and Schwack, 2000). Each of the peppers analysed has a unique profile. Prominent in the yellow pepper profile is the early eluting xanthophyll neoxanthin, and the cluster of non-identi-

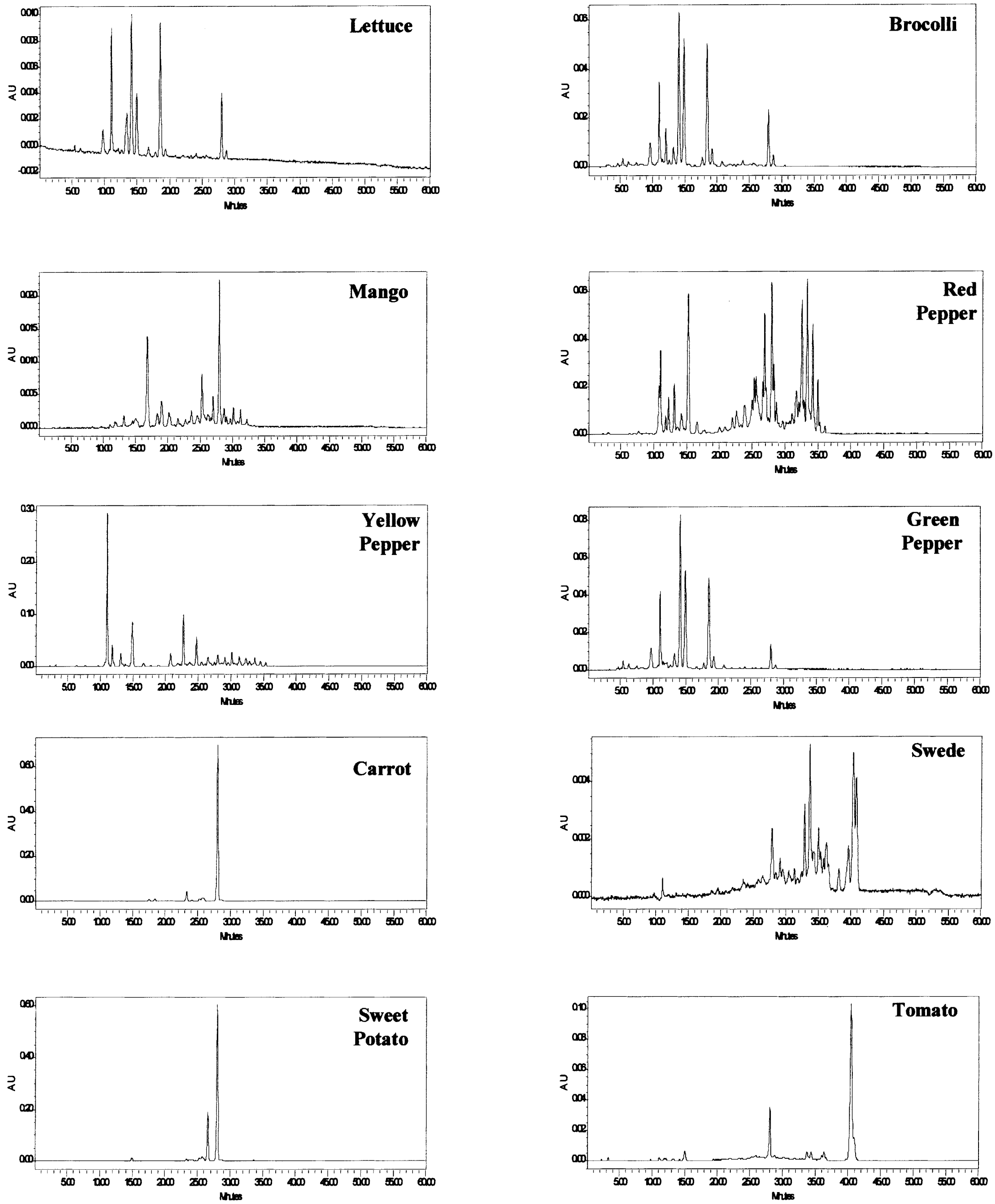

Fig. 2. Pigment profile of fruits and vegetables by RP-HPLC.

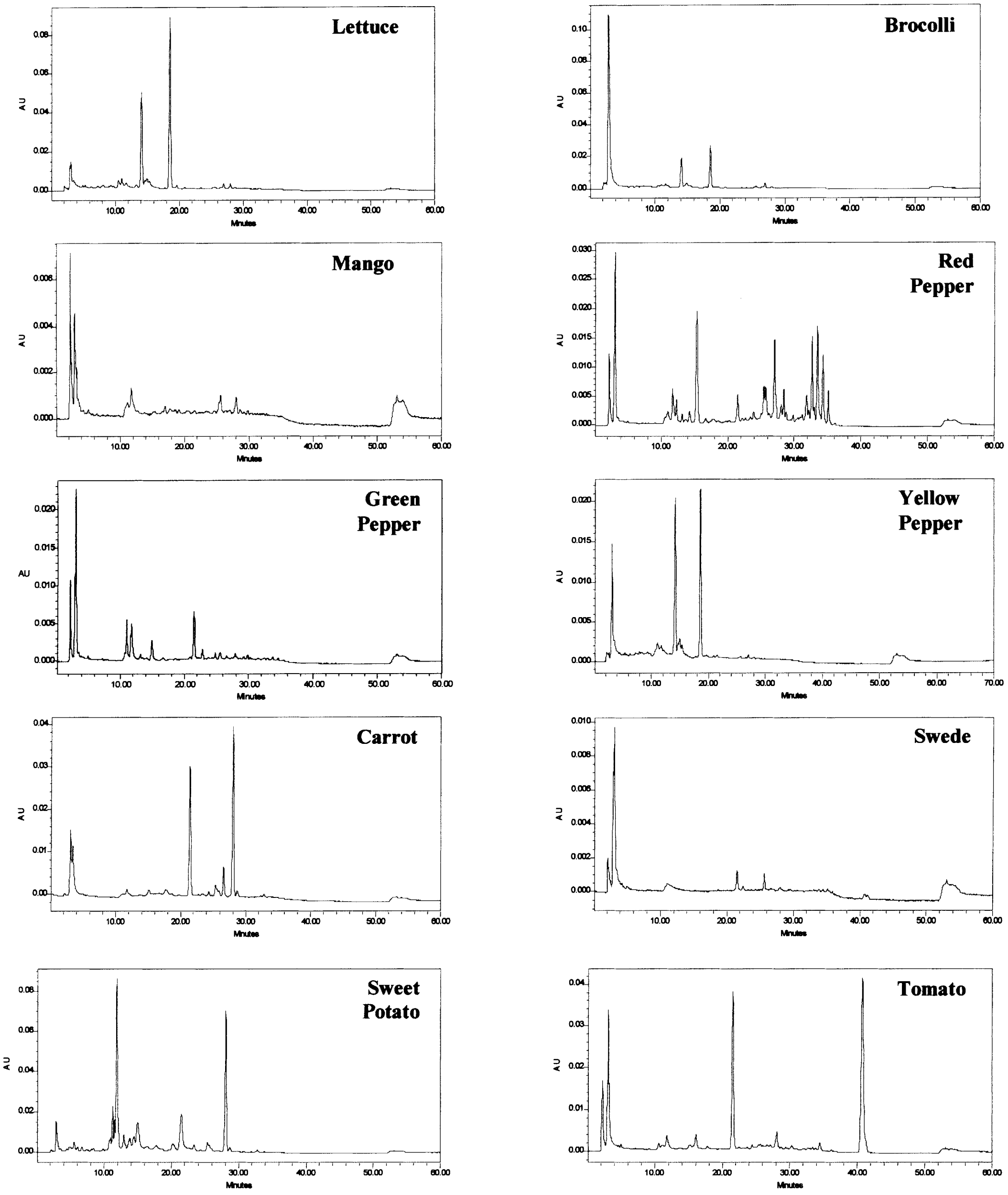

Fig. 3. Profile of tocopherols in fruits and vegetables by RP-HPLC.

fied compounds eluting between 25 and 35 min. On the basis of their retention times and spectral characteristics, these are most likely to be xanthophyll esters, which exhibit identical UV–vis spectra, but are less polar and elute differentially.

β-Carotene and phytoene were conclusively identified in mango, with a cluster of compounds eluting between 25 and 35 min. Carrot, sweet potato and tomato have relatively simple profiles. All *trans* β-carotene is the major carotenoid in both carrot and sweet potato, with carrot also containing high levels of α-carotene. The profile of tomato is well characterized, with lycopene and β-carotene as the major carotenoids. Other than tomato, swede was the only tissue to be analysed which contained lycopene. In addition it also has a cluster of putative xanthophyll esters eluting in the mid portion of the chromatogram.

A number of carotenoids and their isomers could be conclusively identified, and subsequently quantified, on the basis of their retention time, PDA spectra and comparison with the literature (Table 3). On average 77% of carotenoids present in food extracts have been identified and quantified (range 64–98%). The major contributors to the total carotenoid content of foods are β-carotene, lutein, violaxanthin, and when present, lycopene (Table 4).

Table 3
Major carotenoids identified in foods analysed as a percentage of total identified and unidentified

	R_t (min)													
	9.7	11.1	12.3	13.1	15.0	15.2	16.6		22.6	26.6	27.9		40.0	
Produce	Neox.	Violax.	Capsorub.	Anthera.	Lutein	Capsan.	Zeax.	Phyt.	β-Crypt.	α-Carotene	β-Carotene	Cap. ester	Lycopene	Unident.
L	6.5	20.9			15.4						27.2			30.0
B	5.5	12.9			30.2						20.2			31.2
M											56			44
RP		6.1	2.9	4.1		13.95	1.0	0.8	3.2	2.3	20.5	27.1		16.6
YP		24.8			11.6		0.6	5.1		1.5	5.4			51.0
GP		20.3			38.5			0.2			11.0			30
C					0.8			5.2		9.4	84.7			0
S		2.0						37.9					27.0	35.8
SP								3.9			93.5			2.6
T		2.8			10.4			25.8			6.2		57.5	2.1

Neox., neoxanthin; Violax., violaxanthin; Capsorub., capsorubin; Anthera., antheraxanthin; Capsan., capsanthin; Zeax., zeaxanthin; Phyt., total phytoene; β-Crypt., β-cryptoxanthin; Cap.ester, total capsanthin esters; Unident., unidentified carotenoids. L, lettuce; B, broccoli; M, mango; RP, red pepper; YP, yellow pepper; GP, green pepper; C, carrot; S, swede; SP, sweet potato; T, tomato.

Table 4
Content of known carotenoids in fruits and vegetables[a]

Produce	Neox.	Violax.	Capsorub.	Anthera.	Lutein	Capsan.	Zeax.	Phyt.	β-Crypt.	α-Carotene	β-Carotene	Cap. esters	Lycopene	Total
L	51.8±4.7	167.1±14.3			123.9±8.8						225.7±40.4			585.7±61.9
B	0.3±0.1	0.8±0.2			1.9±0.3						1.2±0.2			4.2±0.3
M											49.8±9.8			49.8±9.8
RP	64.8±4.7		31.0±3.2	43.4±3.1		149.2±13.7	11.0±0.3	34.0±10.6	34.2±3.2	25.0±5.7	221.9±53.0	290.5±8.7		878.0±40.9
YP	353.1±65.2				165.6±31.3		9.1±1.7	61.7±13.9	318.5±60.5	20.1±2.5	75.3±11.7			1012.6±179.5
GP		33.5±6.0			64.1±1.8			0.3±0.1			19.0±7.3			116.9±21.3
C					3.5±0.6			36.5±14.0		44.5±9.4	320.5±28.8			408.1±68.0
S		0.1±0.0						1.2±0.3					0.5±0.2	1.5±0.5
SP								16.1±5.2			381.0±51.5			389.3±53.2
T		25.5±5.8			94.9±19.4			234.2±39.1			56.1±13.6		522.5±92.6	908.1±159.7

Neox., neoxanthin; Violax., violaxanthin; Capsorub., capsorubin; Anthera., antheraxanthin; Capsan., capsanthin; Zeax., zeaxanthin; Phyt., total phytoene; β-Crypt., β-cryptoxanthin; Cap. esters, total capsanthin esters; Total, total carotenoids. Abbreviations for produce are shown in Table 3.

[a] Data presented as μg/g dry weight±SEM ($n=4$).

In addition to quantifying the known carotenoids, this approach also separated a number of unidentified carotenoids (Table 5). On-line PDA detection provides absorbance spectra information on the unknown compounds, however the addition of second-generation technology such as MS, MS–MS and MS^n, along with comprehensive spectral databases will undoubtedly provide authoritative information to aid accurate identification of unknowns. It should be noted that MS detection will be unable to distinguish between isomeric forms of carotenoids.

Detection of the eluent at 290 nm reveals the presence of tocopherols in the fruits and vegetables. α- and γ-Tocopherol were detected in low levels in a number of tissues (Table 6), however their presence in the other tissues below the limits of detection (ca. 500 pg/g dry weight) cannot be ruled out. The highest levels of α-tocopherol, (0.6 μg/g dry weight), were detected in red and yellow peppers, compared with only 0.1 μg/g dry weight in green pepper. As tocopherols are also derived from the terpenoid family, alterations in the carotenoid profile can have repercussions on the levels of tocopherols, for example, by metabolic channelling. The green vegetables lettuce, broccoli and green pepper are the only tissues analysed to contain the chlorophylls *a* and *b* (Table 6).

Analysis of plant metabolites and carotenoids in particular, is complicated by their diversity, instability, complexity and the presence of *cis* and *trans* isomers with their diverse spectrum of polarities. These factors have contributed to the incomplete nature of carotenoid food standard tables. However the approach described here, making use of an HPLC C_{30} RP column, is able to optimally separate a great number of carotenoids with negligible sample preparation. Batch extraction of samples can be completed in under an hour, with the HPLC gradient also taking 60 min per sample. Information gained from the analysis of isomers is particularly relevant to studies on carotenoid absorption. Evidence to date supports the theory that

Table 5
Spectral characteristics and occurrence of tentatively identified or unidentified carotenoids

R_t (min)	λ max II	λ max III	Tentative identification	Occurrence
10.81	441.5	469.3	Violaxanthin isomer	RP
11.48	422.2	448.7	UNK[a]	RP, YP
12.05	439.1	468.1	9,9′-*cis*, *cis* Violaxanthin	L
12.66	433.1	459.6	UNK	B, C
13.11	445.1	472.9	UNK	YP, SP
13.21	435.5	463.2	Lactucaxanthin	B, GP
13.45	439.1	469.1	UNK	L
13.67	440.3	469.3	13- or 15-*cis* Violaxanthin	SP
16.74	435.5	463.2	UNK	L
16.83	440.3	469.3	UNK	M
17.54	440.3	469.3	UNK	SP
17.84	439.1	466.9	UNK	L, B, GP
18.36	422.2	447.5	UNK	M, SP
19.02	427.0	445.0	UNK	M
19.34	441.5	468.1	UNK	L, B, GP
20.77	440.3	469.3	UNK	YP
22.00	421.0	447.5	UNK	T
23.31	446.3	474.1	UNK	SP
23.63	441.5	469.3	Violaxanthin ester	M
24.74	441.5	470.5	Violaxanthin ester	YP
25.28	440.3	470.5	Violaxanthin ester	M
26.99	441.5	470.5	UNK	M
27.87		434.3	Poly-*cis* lycopene	S
28.29		475.3	UNK	RP
28.74	446.3	475.3	9-*cis* β-Carotene	L, B GP
29.09	441.5	470.5	Violaxanthin ester	YP
30.12	441.5	470.5	Violaxanthin ester	M
31.27	441.5	470.5	Violaxanthin ester	YP
32.28	441.5	470.5	Violaxanthin ester	YP
32.85	447.5	475.3	Lutein ester	YP
32.89		446.3	UNK	S
33.66	447.5	475.3	Lutein ester	YP
33.70	445.1	469.3	UNK	S
34.49	447.5	475.3	Lutein ester	YP
35.31	447.5	475.3	Lutein ester	YP
38.16		468.1	7-*cis* Lycopene	S

[a] UNK, unknown. See Table 3 for abbreviations of produce.

Table 6
Content of tocopherols and chlorophylls in fruits and vegetables[a]

Produce	α-Tocopherol	γ-Tocopherol	Chlorophyll *a*	Chlorophyll *b*
Lettuce	0.1±0.01	0.3±0.0	1852.7±192.4	1035.4±103.3
Broccoli	0.003±0.001	0.002±0.001	10.6±1.4	8.5±1.4
Mango	0.05±0.01	<LOD		
Red pepper	0.6±0.2	0.1±0.0		
Yellow pepper	0.6±0.2	<LOD		
Green pepper	0.1±0.0	<LOD	412.5±76.0	385.3±72.1
Carrot	0.03±0.01	<LOD		
Swede	<LOD	<LOD		
Sweet potato	<LOD	<LOD		
Tomato	0.2±0.1	0.1±0.0		

<LOD, limit of detection (500 pg/g dry weight).

[a] Data presented as μg/g dry weight±SEM ($n=4$).

the isomeric forms of carotenoids are differentially absorbed (During et al., 2002).

3. Conclusion

The approach described here has enabled investigation of the carotenoid, tocopherol and chlorophyll content and profile of a diverse range of fruits and vegetables (Fig. 4). It offers the advantages of being a robust and established method based on a single run. This approach can be easily applied world-wide to the study of different products and the appraisal of natural and induced variation between samples. A topical application of this approach is in screening genetically modified (GM) and the corresponding non-GM crops for unintended differences in nutrients and metabolites.

Use of on-line PDA detectors in conjunction with the appropriate standards, allows for the routine identification and quantification of major metabolites. The information gained can be used in a number of different applications.

4. Experimental

4.1. Plant material and reagents

Unless stated otherwise, all solvents were HPLC grade, and along with other chemicals were supplied by Merck (Poole, Dorset, UK). Chlorophylls *a* and *b* were supplied by Sigma (Poole, Dorset, UK). All other standards were prepared as previously described (Fraser et al., 2000). Fruits and vegetables were purchased from a local supermarket and stored at room temperature until required. All produce was prepared as described in Table 2.

4.2. Extraction of carotenoids

Carotenoids in powdered freeze-dried tissue were extracted using the method described by Fraser et al., (2000). In brief, after the addition of 100 μl MeOH samples (10 mg) were vortexed for 5 min prior to the addition of 100 μl Tris–HCl (50 mM, pH 7.5 containing 1 mM NaCl). Samples were mixed and left on ice for 10 min.

Carotenoids were then extracted into 400 μl CHCl3 during a 10 min incubation on ice. The organic phase was separated by centrifugation at 3000 rpm and removed. The aqueous phase was then re-extracted with $CHCl_3$. The organic phases were pooled and dried down under nitrogen.

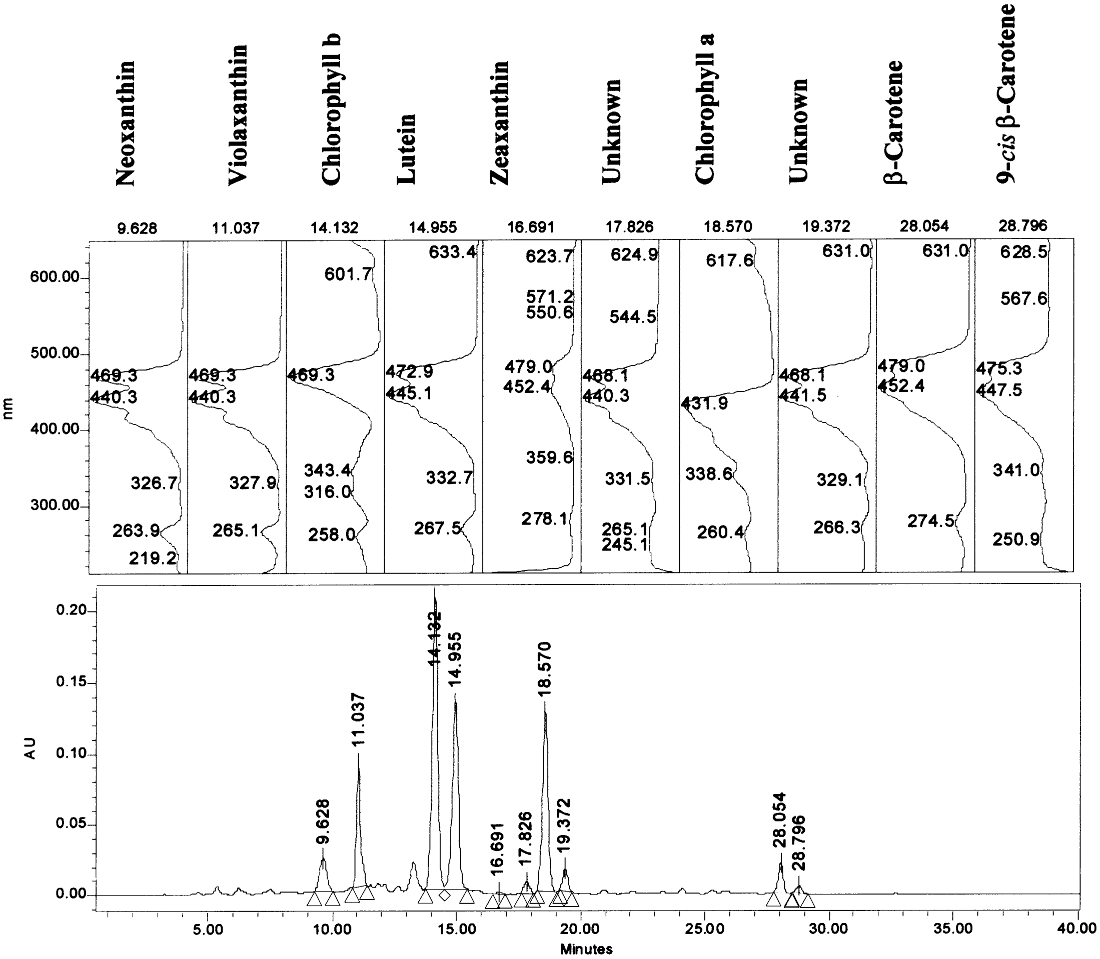

Fig. 4. Elution profile and PDA spectra of xanthophylls, carotenes, tocopherols and chlorophylls from green pepper.

4.3. Separation and identification of metabolites

Tissue extracts were reconstituted in EtOAc and analysed using a Waters Alliance 2600S HPLC with diode array monitoring between 200 and 600 nm. Isoprenoids were separated using a RP C_{30} 5 μm column (250×4.6 mm) coupled to a 20×4.6 mm C_{30} guard column (YMC Inc., Wilmington, NC, USA) operating at 25 °C and eluted with a 1 ml/min gradient of (A) MeOH, (B) 20% aq. MeOH, containing 0.2% ammonium acetate and (C) *tert*-butyl methyl ether (Fraser et al., 2000). In brief, the isocratic elution was 95% A, 5% B for 12 min, a step to 80% A, 5% B and 15% C at 12 min, followed by a linear gradient to 30% A, 5% B and 65% C at 30 min. The injection volume was 10 μl. The column was returned to the initial conditions and equilibrated over 30 min. Standards of all *trans* β-carotene, α-carotene, lycopene, capsanthin, β-cryptoxanthin, lutein and phytoene were prepared and used to identify and quantify (using maxplot detection) both known and related unknown carotenoids. Capsorubin was quantified as capsanthin equivalents, while all other carotenoids (e.g. violaxanthin and neoxanthin) were quantified as lutein equivalents. γ-Tocopherol was quantified as α-tocopherol equivalents (at 290 nm).

References

Block, G., Patterson, B., Sauber, A., 1992. Fruit, vegetables and cancer prevention: a review of epidemiological evidence. Nutrition and Cancer 18, 1–29.

Breithaupt, D.E., Schwack, W., 2000. Determination of free and bound carotenoids in paprika (*Capsicum annuum* L.) by LC/MS. European Food Research Technology 211, 52–55.

Britton, G., Liaanen-Jensen, S., Pfander, H., 1995. Isolation and analysis. In: Britton, G., Liaanen-Jensen, S., Pfander, H. (Eds.), Carotenoids, Vol. 1A. Birkhäuser, Basel, pp. 13–15.

During, A., Hussain, M.M., Morel, D.W., Harrison, E.H., 2002. Carotenoid uptake and secretion by CaCo-2 cells: β-carotene isomer selectivity and carotenoid interactions. Journal of Lipid Research 43, 1086–1095.

Fiehn, O., Kopka, J., Dormann, P., Altmann, T., Trethewey, R.N., Willmitzer, L., 2000. Metabolite profiling for plant functional genomics. Nature Biotechnology 18, 1157–1161.

Fraser, P.D., Römer, S., Shipton, C.A., Mills, P.B., Kiano, J.W., Misawa, N., Drake, R.G., Schuch, W., Bramley, P.M., 2002. Evaluation of transgenic tomato plants expressing an additional phytoene synthase in a fruit specific manner. Proceedings of the National Academy of Sciences of the United States of America 99, 1092–1097.

Fraser, P.D., Pinto, M.E.S., Holloway, D.E., Bramley, P.M., 2000. Application of high performance liquid chromatography with photodiode array detection to the metabolic profiling of plant isoprenoids. The Plant Journal 24, 551–558.

Giovannucci, E., 1999. Tomatoes, tomato-based products, lycopene and cancer: a review of the epidemiological literature. Journal of the National Cancer Institute 91, 317–331.

Grassmann, J., Hippeli, S., Elstre, E.F., 2002. Plant's defence mechanism and its benefits for animals and medicine: role of phenolics and terpenoids in avoiding oxygen stress. Plant Physiology and Biochemistry, 40, 471–478.

Holden, J.M., Eldridge, A.L., Beecher, G.R., Buzzard, I.M., Bhagwat, S.A., Davis, C.S., Douglass, L.W., Gebhardt, S.E., Haytowitz, D.B., Schakel, S., 1999. Carotenoid content of US foods: an update of the database. Journal of Food Composition and Analysis 12, 169–196.

Kritchevsky, S.B., 1999. β-Carotene, carotenoids and the prevention of coronary heart disease. Journal of Nutrition 129, 5–8.

Landrum, J.T., Bone, R.A., 2001. Lutein, zeaxanthin and the macular pigment. Archives of Biochemistry and Biophysics 385, 28–40.

Lessin, W.J., Catigani, G.L., Schwartz, S.J., 1997. Quantification of cis-trans isomers of provitamin A carotenoids in fresh and processed fruits and vegetables. Journal of Agricultural and Food Chemistry 45, 3728–3732.

Mayne, S.T., 1996. β-Carotene, carotenoids and disease prevention in humans. FASEB Journal 10, 690–701.

Römer, S., Fraser, P.D., Kiano, J.W., Shipton, C.A., Misawa, N., Schuch, W., Bramley, P.M., 2000. Evaluation of the provitamin A content of transgenic tomato plants. Nature Biotechnology 18, 666–669.

Sander, L.C., Sharpless, K.E., Craft, N.E., Wise, S.A., 1994. Development of engineered stationary phases for the separation of carotenoid isomers. Analytical Chemistry 66, 1667–1674.

van Poppel, G., Goldbohm, R.A., 1995. Epidemiological evidence for β-carotene and cancer prevention. American Journal of Clinical Nutrition 62, 1493–1503.

Ye, X., Al-Babili, S., Klöti, A., Zhang, J., Beyer, P., Potrykus, I., 2000. Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287, 303–305.